Beiträge zur Cadmiumwirkung auf den Hühnerembryo
Histologische, histochemische und autoradiographische Untersuchungen

INAUGURAL-DISSERTATION
zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Universität Düsseldorf

vorgelegt von

WING-KEUNG LIU

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Referent: Prof. W. Hilscher
Korreferent: Prof. G. Willuhn

Toxicity of cadmium chloride on the chick embryos :
A histological, histochemical and autoradiographic study

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Summary

The toxicity of cadmium was studied on the developing chick embryos from the 5th to the 8th day of incubation. Two administration methods, namely the air chamber and the window injection method, were employed.

Cadmium chloride solution at concentrations of 0.1, 0.5 and 1.0 ppm was injected into the air chamber of the egg before incubation. The mortality rate of the chick embryos increases significantly with respect to the increase of the cadmium concentration and the days of incubation. The 0.1 ppm $CdCl_2$ is too low a concentration to cause any significant effect on the embryo. Investigation groups show that $CdCl_2$ has influences on the histochemistry of the embryos, but not on the histology of the organs :

1. The mitotic index and the autoradiographic study indicate that cadmium depresses the proliferative activity of the liver and the mesonephros. This phenomenon is especially prominent between the 5th and the 6th day of incubation, when the mitotic activity is at its maximum.
2. The mesonephros of the cadmium treated embryo loses its normal functions. The ability to retain trypan blue granules by the proximal tubules cells decreases significantly in the embryos administered with 0.5 ppm $CdCl_2$. No trypan blue granule can be found in the mesonephroi of the 1.0 ppm $CdCl_2$ embryos.
3. Enzyme activities of the acid phosphatase and the lactic dehydrogenase increases slightly in the mesonephroi, but increase slightly in the livers of the cadmium treated embryos.

4. Except part of the 7-day old embryos administered with 1.0 ppm $CdCl_2$, no prominent histological alteration could be observed in the organs of all the cadmium treated survivors.

5. Analysis by the atomic absorption spectrometry shows that a great variation of cadmium content can be found in the embryos from the same groups in the air chamber injection method. Embryos with a considerable amount of cadmium in the bodies died, and those alive and with functional and histochemical changes in the mesonephroi possessed a relatively low cadmium content. Some of them even had the same cadmium level as the controls. This may account for the few prominent histological variations in the organs in the air chamber injection method.

When the $CdCl_2$ solution from 0.01, 0.05 and 0.1 ppm was applied supravitellinely onto the 1-day old chick embryo through a window in the shell, a more drastic mortality rate was found among the chick embryos with a lower range of $CdCl_2$ solution when compared to the air chamber injection method. No alive embryo was found in the 0.1 ppm $CdCl_2$ treated groups after the 6th day of incubation. Prominent variations could be observed in the embryo both in the histological and histochemical aspects :

1. Gross morphological changes were observed in the embryos with $CdCl_2$ administered supravitellinely. Defected brain and eyes, distorted body shape were common among the retarded developed embryos.

2. Severe histological alterations in the spinal cord, eyes, pancreas, liver and mesonephros were found early in the 5-day old embryos.

3. Enzyme activities of the acid phosphatase and the lactic dehydrogenase increase in the mesonephroi, but decrease in the livers of the cadmium treated embryos.

From 1. The histological alterations in the spinal cord, eyes, pancreas, liver and mesonephros,
2. the loss of the retaining ability of trypan blue granules by the mesonephric proximal tubular cells, as well as
3. the change of enzyme activities of the acid phosphatase and the lactic dehydrogenase in the liver and the mesonephros,

the defect of the functioning mesonephros appears to be closely related to the cadmium content and also to be the major lethal factor of the cadmium poisoned chick embryos.

I. Introduction

Cadmium, a silver white metal, is recovered from dust and precipitates collected in zinc or lead smelters. Though it is only a by-product, cadmium is widely used in electro-plating, pigments, plastic stabilizers, alloys, fungicides and in control elements in nuclear reactors. Its world production has increased from 14 tons in 1910 to 13.700 tons in 1974, and during the past decade, annual production has been stable at 10,000 to 14.000 tons (Stubbs, 1978).

Cadmium occurs naturally as an isomorph impurity in zinc or lead ores. Through weathering or erosion only an insignificant amount will enter our ecosystem. Accompanying its increasing world consumption, large amounts of cadmium get into the waterways from industrial wastes, or into the atmosphere from smelter fumes as well as from combustion of coal and oil. According to an investigation of the National Inventory (1968), nearly one third of the cadmium used by industries was emitted into the environment as a pollutant. Surveys showed that about 23.5 ppm of cadmium remained in the soil in an area of a dust fall-out in New Brunswick, Canada (Dugdal, 1977); about 57.4 ppm in river sediment and 9.3 ppm in the irrigating canal in Ohsujizewa of Japan (Yamagata, 1975); and more than 100 ppm in sludge from the sewage works in England (Berrow & Weber, 1972). Once the cadmium has entered the food chain through the plants or the marine organisms, it remains in organisms and can produce progressive chronic diseases, such as interference with mineral metabolism, cell mitochondrial respiration, and photosynthesis in plants and hypoxia in fishes. In mammals, chronic cadmium intoxication can lead to anemia, retardation of testicular development, enlargement of joint, scaly skin, liver and kidney damages, reduced growth and increased mortality.

The cadmium poisoning had not aroused any public attention until the official announcement of the "Itai-itai" disease around the Jinzu River in Japna, which was caused by cadmium (Japan Ministry of Health and Welfare 1968). A special committee from the International Lead Zinc Research Organization was formed concerning this environmental hazard, followed by the establishment of the Cadmium Council in the USA, the International Cadmium Institute in Europe, and the "Itai-itai" Disease committee in Japan. Many regulations and environmental laws have been recommanded by countries which ultilize cadmium : 5 ug/l of cadmium is the maximal admissible concentration in drinking water (Commission of the European Committee, 1976); 0.5 ug/m^3 in ambient air (WHO, 1974) and between 400 - 500 ug the maximal weekly uptake by man (FAO/WHO, 1972). In 1977, a world wide organization, the Cadmium Association, was established to investigate the toxicity of cadmium in order to improve its production technology and to solve the health problems caused by it. A conclusion of its 1st International Conference was that more reliable bioassays of cadmium were necessary (Hendrick, 1978). Since then only limited investigations have studied the effects of cadmium on domestic poultry, especially on the developing chick embryos.

Existing information is that most of the injected radioactive cadmium can be traced subsequently in the kidneys and liver of the chick (Sell, 1975). The chick testis showed no significant morphological changes under cadmium treatment (Ericksson and Pinus, 1964). Activities of certain enzymes from the cadmium poisoned chick have been reported by Kench and Gibb (1970). The LD_{50} and the histological observations from the combined effects of Cd and Zn have been investigated in the developing chick embryos (Birge and Roberts, 1976; Ribas and Schmidt, 1973).

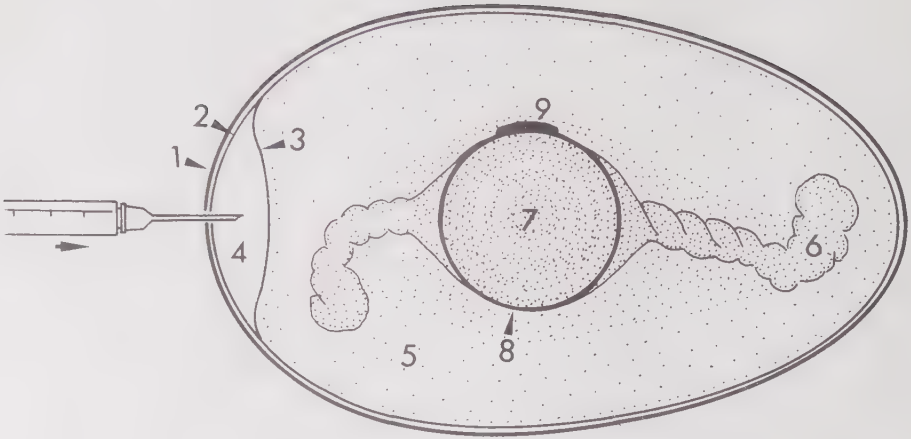
The present research work was carried out in order to study the effects of cadmium on the early stages of organ development in chick embryos, focusing on the liver and the mesonephros, which are known to be highly sensitive to cadmium in mammals. Two methods of cadmium administration into the chick embryos were employed, and a comparison is made between the effects from the air chamber injection method to that of the window injection method.

II. Materials and Methods

A. Air chamber injection method

1. Survival rate and body weight

White Leghorn chick eggs (Lehmann Tierzucht, Germany) were injected with cadmium chloride solution on the first day before incubation. The blunt end of the egg was swabbed with 70% alcohol and a hole was punched by a sterilized needle. 0.1 ml $CdCl_2$ in sterilized physiological saline was injected through the hole into the air chamber. The amount of $CdCl_2$ was adjusted to provide a concentration in the egg of 0.1, 0.5 or 1.0 ppm. The concentration of the cadmium solution was based on the measurement of the average weight of 1000 eggs. During the injection, care was taken so as not to break through the inner shell membrane (Fig. 1). After the injection, the hole was sealed with plaster (Leukoplast, Hamburg, W. Germany). The entire injection procedure was carried out in laminar air flow chamber (BH-NG, Gilman Instrument, Italy). Intact eggs and those receiving 0.1 ml sterilized physiological saline were used as control (control and saline control). The eggs were incubated at a temperature of $37^{\circ}C \pm 0.5^{\circ}C$ and a relative humidity of $65 \pm 3\%$ (Freeman and Vince, 1974). The embryos were collected from the 4th to the 9th day of incubation, and weighed. Survival rate and average body weight were noted. The embryos from the 5th to the 8th day were fixed in 4% formalin for paraffin sections.



1 shell
2 outer shell membrane
3 inner shell membrane
4 air chamber
5 albumin
6 chalaza
7 yolk
8 vitelline membrane
9 blastoderm

Fig. 1. Air chamber method

2. Histological observations

The formalin fixed and paraffin embedded chick embryos were cut into 4 µ section and stained with Haematoxylin and Eosin for histological observation (Humason, 1967).

3. Enzyme histochemistry

Cd-treated and controls chick embryos (five embryos per group) from the 5th to the 8th day were collected and fixed in cold formaldehyde (4°C) for 12 hours and then transferred to cold gum-sucrose (4°C) for a further 12 hours (Lodja, 1976). The embryos were removed and carefully dried with filter paper in order to remove any excess gum-sucrose and then frozen in liquid nitrogen. Sections of 5 µ thick were cut with a cyrostat (NW 1832, Bright Co., England) at -25°C and the activities of acid phosphatase and lactic dehydrogenase determined.

Acid Phosphatase (ACPase)

Using the azo dye technique, sections were incubated at a temperature of 37°C for 90 minutes with α-naphthyl-phosphate as basic substrate and hexaazotised pararos-aniline as the coupling agent. The reaction was carried out at a pH 5.5 (Barka and Anderson, 1962).

Lactic dehydrogenase (LDH)

Using the tetrazolium technique, sections were incubated at a temperature of 37°C and a pH 7.2 for 90 minutes with Na-lactate as the substrate and nicotinamide adenine-dinucleotide as the co-enzyme (Lodja et al., 1976).

4. Proliferation rate

a. Mitotic index

The mitotic index of the liver and the mesonephros was based on 5000 cell counts per embryo from the sections stained with haematoxylin and eosin. Five embryos were employed in each group.

b. Autoradiography

Chick embryos from treated and control groups of the 5th to the 8th day were collected from the incubator, and a window was made in the egg shell (Rugh,1977). 20 /u Ci ^{3}H-thymidine (Sp. act. 5 Ci/m-mole, Radiochemical Center Amersham, England) dissolved in 0.1 ml sterilized physiological saline was injected into each yolk sac through the window. After injection, the window was sealed with a plaster and replaced into the incubator for one hour incubation. The embryos were then collected, fixed in Bouin's fluid and embedded in paraffin. Sections (4 /u thick) were coated with nuclear track emulsion (Kodak Co., Germany) and kept in the dark at 4°C for 6 - 10 days, depending on the age of the chick embryos. After the exposure time, sections were developed, fixed amd stained with haematoxylin. Thymidine, a specific precursor of DNA, is normally incorporated during the S-phase of the dividing cells. The silver grains in the nuclei of 5000 hepatic and mesonephric tubular cells per embryos were counted. Five embryos were used in each group.

5. Mesonephros functional test

Four groups of 15 eggs (control, saline control, 0.5 ppm and 1.0 ppm $CdCl_2$) were injected into each air chamber with 0.7 ml trypan blue solution (Bayer Co., Germany) at the end of the 4th incubation day. 1.0% trypan blue was dissolved in sterilized physiological saline. After in-

jection, the eggs were further incubated for 24 hours. At the end of this incubation period, the embryos were collected and fixed in Bouin's fluid for paraffin sectioning (Hurd, 1928). Similar groups were prepared for the test of the mesonephros after 5 to 8 days of cadmium administration, but 1.0 ml of trypan blue solution was injected into the enlarged air chamber.

Paraffin sections of 4 μ thick were stained with Kernechtrot, or were simply deparaffinized and mounted with Eukitt(R) (Klees, Germany). The number of cells with trypan blue granules were counted in 5000 mesonephric proximal tubular cells from each embryo.

B. Window injection method

The White Leghorn chick eggs (Lehmann Tierzucht, Germany) were incubated under the same condition as in the air chamber injection method for one day. On the second day (i.e. after 24 hours), each egg was candled and the position of the blastoderm was marked on the shell of the egg with a pencil. The egg was placed horizontally in a finger bowl, and the blunt end and the upper surface of the shell, including the area of the blastoderm, was swabbed with 70% alcohol. A window of 0.5 cm x 0.5 cm was made on the egg shell using a sterilized saw blade (Fig. 2A). A hole was punched at the blunt end with a sterilized needle. After 1 ml of the albumin was withdrawn from this hole (Fig. 2B), 0.9 ml of Locke's solution together with 0.1 ml of $CdCl_2$ solution (ranging from 0.01 ppm, 0.05 ppm and 1.0 ppm based on the average egg weight) was administered through the window on the blastoderm (Fig. 2C). The window and the hole were closed with plaster and the eggs were placed into the incubator again for further development (Rugh, 1977; Christ, 1979 - private communication). On the 5th to

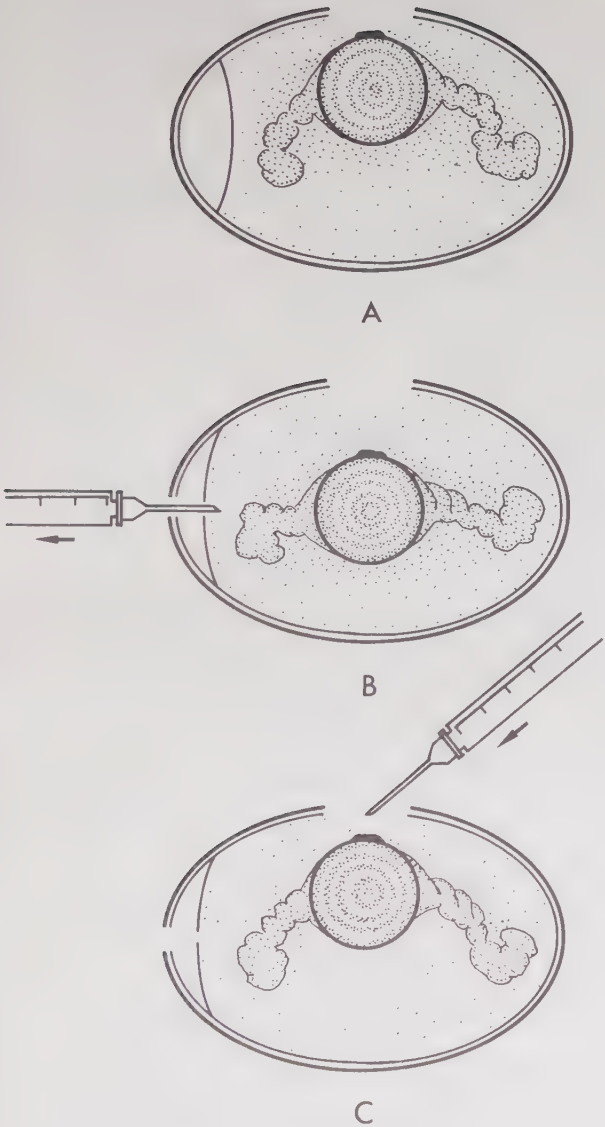


Fig. 2. Window method

the 8th day, the eggs were broken and the malformations of the embryos were noted, photographied and fixed in 4% formalin for paraffin section and histological observation.

Haematoxylin and Eosin staining for the histological observation and activities of ACPase and LDH for the enzyme histochemistry were made in the embryos from the window injection method.

C. Statistical method

Student t-test was used to compare the significance of difference between the groups after cadmium treatment and the controls. For the comparison of the survival rate, Kullback Test was employed.

III. Results

A. Air chamber injection method

1. Survival rate and body weight

The percentage of survival embryos from the air chamber injection method are shown in Tab. 1 and Fig. 3. The measurement was based on a sample size of 50 eggs per group (except in the trial groups on the 4th and the 9th incubation day where only 10 eggs were employed in each group). Analysis of the mortality rate showed that the effects of day of injection and the cadmium concentration (0.5 ppm and 1.0 ppm) were significant ($p < 0.001$).

The survival rate decreases as the embryos develop. The same phenomenos is also found in the controls. Mortality remained steady at 15 to 20% after 7 incubation days. The 0.1 ppm groups shared a similar survival pattern, and showed no significant difference from controls. On the other hand, a larger dose of cadmium resulted in an earlier significant mortality. The percentage survival decreases drastically from 64.7% on the 5th day to that of 20.9% on the 8th day.

Significant decreases in body weight ($p < 0.05$) were found in all the groups that had 1.0 ppm $CdCl_2$, and two groups from those that received 0.5 ppm $CdCl_2$ (i.e. the 6th and the 8th day). The embryos with 0.1 ppm possessed similar body weight when compared with the saline controls (Tab. 2).

2. Histological observation

No gross morphological changes could be observed from the survivors that had cadmium injected into their air

Table 1. The survival rate (%) of chick embryos (Air chamber method)

Groups \ Days	4	5	6	7	8	9
Control	100▲	100▽	100	87.2	86.5	84.5
Saline	100	93.9	92.5	83.7	80.0	80.0
0.1 ppm $CdCl_2$	100	91.2	83.8	81.5	75.0	70.0
0.5 ppm $CdCl_2$	100	91.5	69.4	75.6	44.4	33.3
1.0 ppm $CdCl_2$	92.3	64.7	56.3	35.1	20.9	23.1

▲ The survival rate on the 4th and the 9th day is based on 10 embryos per group.
▽ The survival rate from the 5th to 8th day is based on 50 embryos per group.

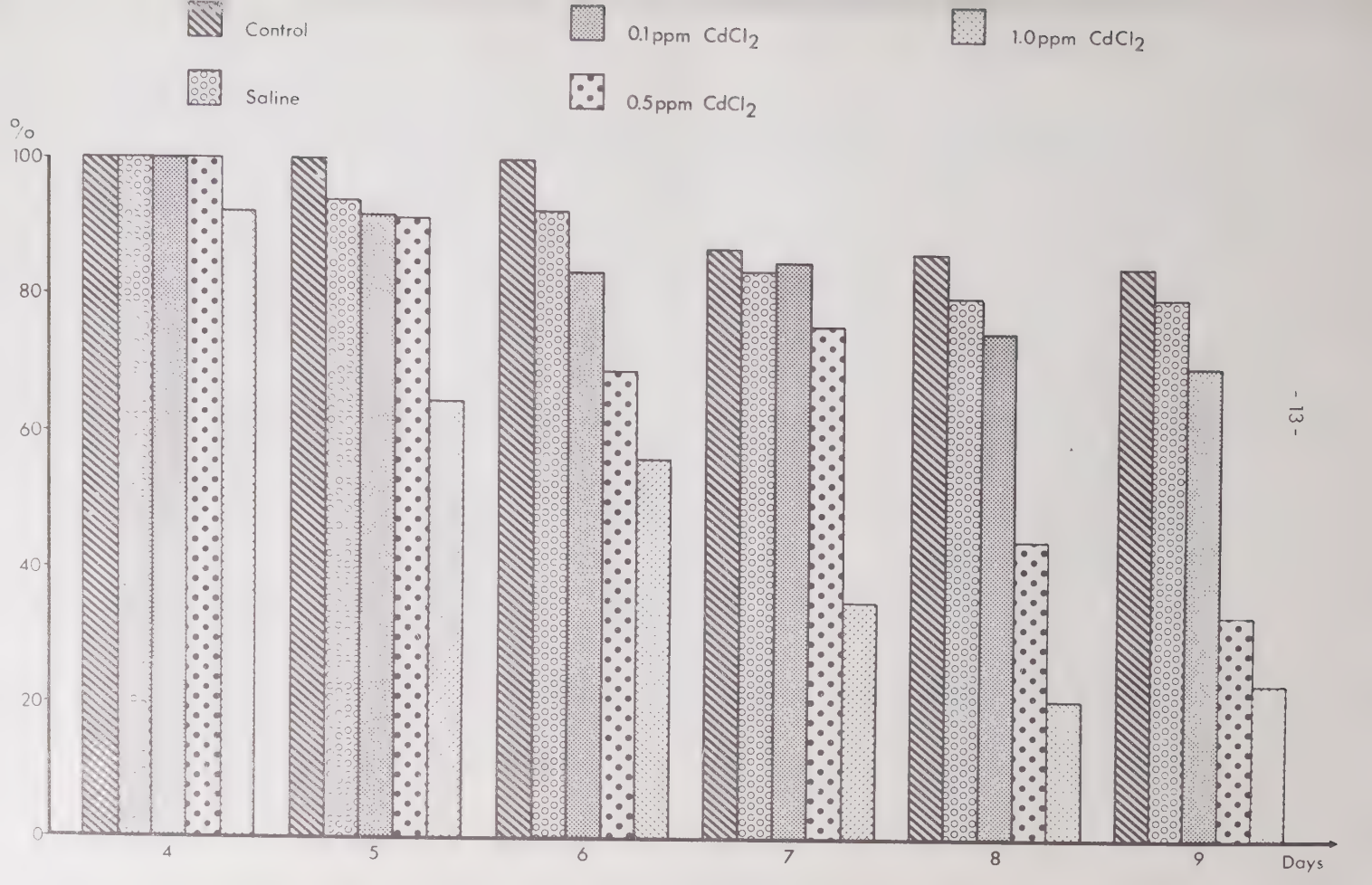

Control
Saline
0.1ppm $CdCl_2$
0.5ppm $CdCl_2$
1.0ppm $CdCl_2$
%
100
80
60
40
20
0
4
5
6
7
8
9
Days

Table 2. The body weight of chick embryos (Air chamber injection method)

Groups \ Days	5	6	7	8
Control	0.11 ± 0.04▲	0.27 ± 0.08	0.43 ± 0.02	0.95 ± 0.22
Saline	0.10 ± 0.05	0.24 ± 0.07	0.43 ± 0.20	0.94 ± 0.23
0.1 ppm $CdCl_2$	0.11 ± 0.05	0.22 ± 0.08	0.42 ± 0.24	0.97 ± 0.23
0.5 ppm $CdCl_2$	0.10 ± 0.03	0.22 ± 0.07*	0.42 ± 0.02	0.80 ± 0.27*
1.0 ppm $CdCl_2$	0.08 ± 0.04*	0.20 ± 0.07*	0.30 ± 0.18*	0.60 ± 0.31**

▲ Body weight (m ± S.D.) is in unit of gram and is from 50 embryos per group.

* $p < 0.05$

** $p < 0.01$

chambers for 5 to 8 days (Fig. 14).

The histology of the target organs of cadmium toxicity, namely the pancreas, the liver and the mesonephros, appeared normal in all groups when compared with the controls (Fig. 21, 23, 26), except in the embryos which had 1.0 ppm $CdCl_2$ administration for 7 days. Some of these embryos showed hypertrophy of the hepatocytes with vacuolization caused the destruction of the liver structure and prominent enlargement of the tubules in the mesonephros, including the proximal and distal tubules (Fig. 27).

3. Enzyme histochemistry

a. Acid phosphatase (ACPase)

In the liver of the controls (Fig. 30), large granules strongly positive for ACPase were scattered through the whole cytoplasm of both parenchyma and Kupffer cells. No change of activity within 5 - 8 days was observed.

ACPase activity was highest in the cytoplasm of the convoluted tubules, especially along the luminal border. The quantity of enzyme diminished towards the outer border. The ACPase was moderate positive in the collecting tubules and very mild in the glomeruli. In the normal mesonephros, the ACPase was arranged in fine granules or dots. This distribution of enzyme activity appeared after the 5th incubation day and showed no distinct change up to the 8th day (Fig. 32).

The ACPase activity decreased slightly in the liver parenchyma and was arranged either in dots or large granules after administration of $CdCl_2$ in the air chamber. On the other hand, the ACPase activity in the tubular cells increased slightly and appeared as large granules. The enzyme activity became more concentrated as the cadmium level was elevated.

b. Lactic dehydrogenase (LDH)

The LDH activity in liver parenchyma was strongly positive and appeared in a cap shape in the cytoplasm (Fig. 34).

An intense finely granulate LDH reaction occured along the luminal border of all the tubular cells - proximal, distal and collecting tubules. An intensity gradient was found declining from the inner border towards the outer border of the tubular cells (Fig. 36).

Some of the liver cells had the same LDH activity as the control, but generally a decrease of LDH activity was observed among the parenchyma of the cadmium treated embryos.

The mesonephros of the cadmium treated embryos showed a slight increase in LDH activity, especially in the tubular cells. The enzyme reaction was found along the inner border or the apical region of the tubular cells. Glomeruli had the same enzyme activity as the controls.

4. Proliferation rate

a. Mitotic index

The mitotic index of the 0.1 ppm groups was not significantly different from the saline controls. In contrast to this lowest concentration, the mitotic index decreased significantly in the liver (Tab. 3 and Fig. 4) and the mesonephros (Tab. 4 and Fig. 5) of the 0.5 ppm and 1.0 ppm experimentals from 5 to 8 days. Maximal mitotic activity

Table 3. Mitotic index (o/oo) in the liver of chick embryos (Air chamber method)

Groups \ Days	5	6	7	8
Control	56.2 ± 10.6 ▲	29.6 ± 2.2	21.8 ± 4.2	14.8 ± 1.9
Saline	45.4 ± 5.8	26.8 ± 3.2	21.6 ± 2.8	14.6 ± 1.6
0.5 ppm $CdCl_2$	39.8 ± 3.6***	23.0 ± 3.2***	18.4 ± 2.8***	11.0 ± 2.2***
1.0 ppm $CdCl_2$	36.6 ± 8.4**	19.0 ± 3.2***	15.2 ± 3.8***	9.7 ± 2.1***

▲ M ± S.D. of the hepatic mitotic index from 5 embryos per group

** $p < 0.01$

*** $p < 0.001$

Fig. 4. Mitotic index in the liver of the chick embryo

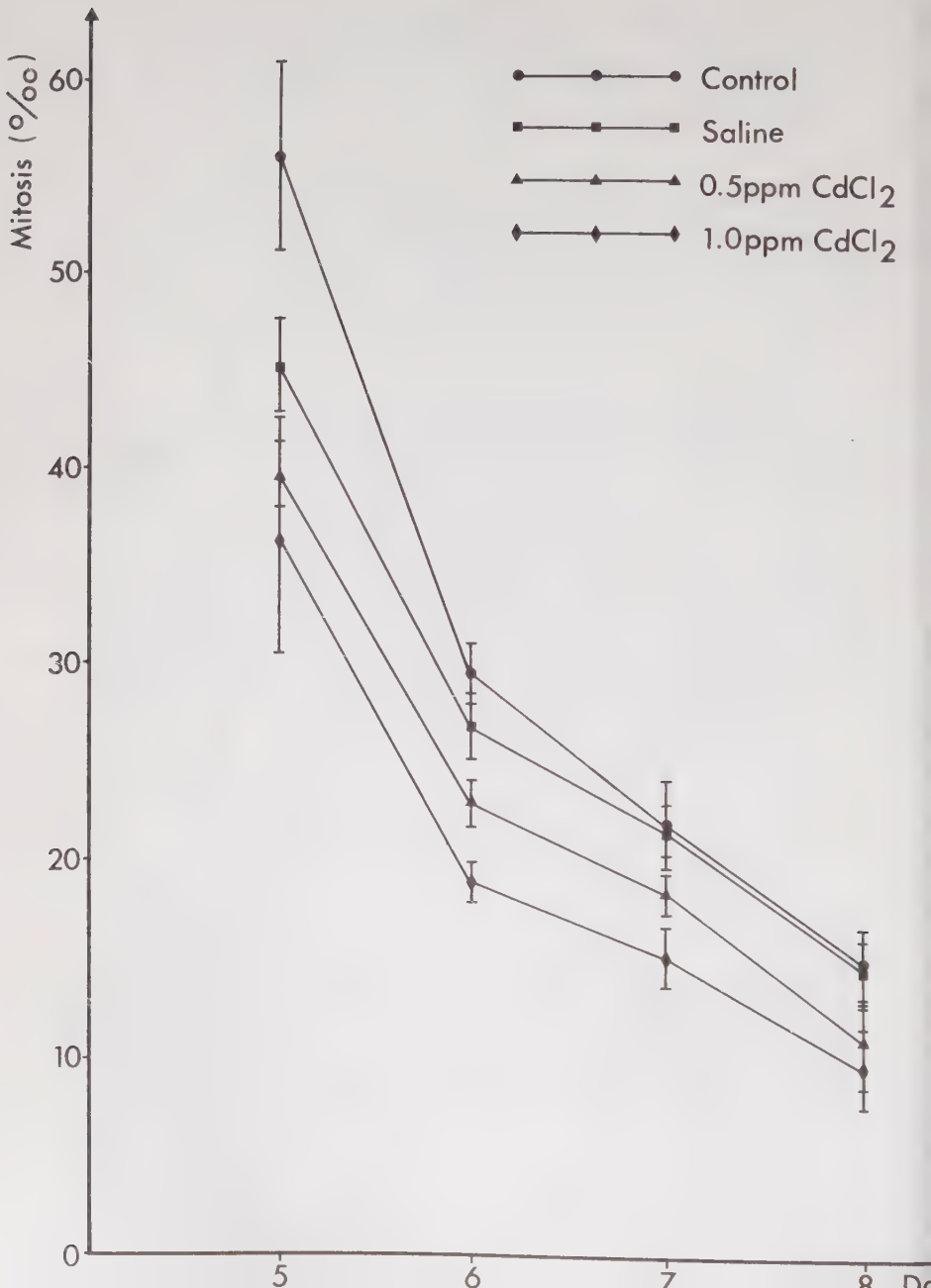


Table 4. Mitotic index (o/oo) in the mesonephros of chick embryos (Air chamber method)

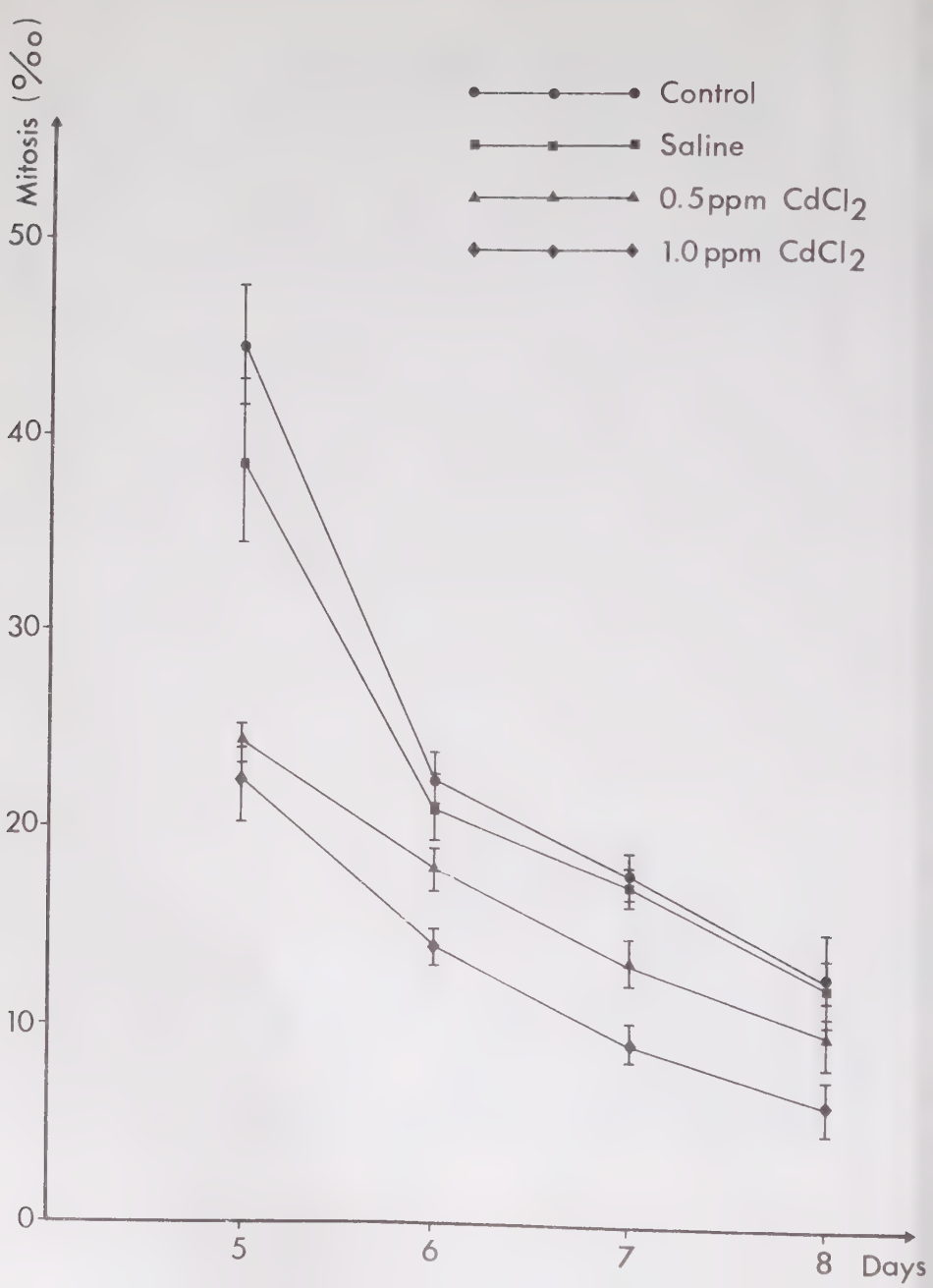
Groups \ Days	5	6	7	8
Control	44.6 ± 8.2▲	22.4 ± 2.0	17.8 ± 2.0	12.6 ± 2.4
Saline	38.6 ± 8.8	21.2 ± 2.2	17.2 ± 2.4	12.2 ± 1.5
0.5 ppm $CdCl_2$	24.2 ± 1.9***	18.0 ± 3.0**	13.2 ± 3.0***	9.9 ± 1.7**
1.0 ppm $CdCl_2$	22.4 ± 3.8***	14.0 ± 3.2***	9.4 ± 2.6***	6.2 ± 1.5***

▲ M ± S.D. of mesonephric mitotic index from 5 embryos per group

** $p < 0.01$

*** $p < 0.001$

Fig. 5. Mitotic index in the mesonephros of the chick embryo



in the liver and the mesonephros was found on the 5th day and then declined with age.

b. Autoradiography

The labelling indices of the liver and the mesonephros are presented in Tables 5 and 6. Positive labellings are taken when three or more silver grains are present in the nucleus (Fig. 6-9). In the columns with 3 - 9 and $\geq$10 grains per nucleus, the labelling decreased with age, especially between the 5th and the 6th incubation day (Fig. 10 - 11). Within those groups from the same incubation day, the labelling index between the experimentals and the controls showed significant differences ($p < 0.001$).

5. Mesonephros functional test

An average of 50% mortality was found in the trypan blue treated embryos. Traces of the trypan blue could be observed on the 5th day. In addition, the mesonephros also showed a pale green colour, but the colour intensity was not strong enough for microscopic investigation until from the 7th day.

The fine granules of the trypan blue were seen along the basal membrane of the columnar cells of the proximal tubules (Fig. 12). A decreasing anterior-posterior gradient was found in the mesonephros. All the groups that received cadmium showed significant decrease in trypan blue amounts in the proximal tubules when compared to the controls ($p < 0.001$) (Table 7). The mesonephroi from the highest dosage group do not take up any trypan blue on the 7th day, and the embryos died from the 8th day (Fig. 13).

Table 5. The labelling index (o/oo) in the liver (Air chamber injection method)

Days	Grain count / Groups	0-2	3-9	⩾10
5	A	560.5 ± 26.5 ▲	233.5 ± 24.5	206.0 ± 25.7
	B	618.6 ± 19.5	192.2 ± 15.7	189.2 ± 27.2
	C	683.4 ± 43.8	179.8 ± 18.5 ***	136.8 ± 34.7 ***
	D	731.6 ± 53.0	168.4 ± 23.5 ***	100.0 ± 28.4 ***
6	A	751.2 ± 30.2	145.3 ± 18.1	103.5 ± 16.8
	B	783.4 ± 42.5	115.4 ± 23.8	101.2 ± 20.4
	C	822.4 ± 44.0	90.4 ± 9.3 ***	87.2 ± 15.1 ***
	D	851.8 ± 10.1	67.0 ± 10.6 ***	81.2 ± 11.1 ***
7	A	822.2 ± 20.6	96.4 ± 12.6	80.8 ± 7.7
	B	830.1 ± 56.0	95.6 ± 12.3	74.3 ± 12.2
	C	824.6 ± 38.9	118.0 ± 14.4 ***	57.4 ± 13.0 ***
	D	865.2 ± 18.8	82.7 ± 23.5 ***	52.1 ± 11.0 ***
8	A	856.2 ± 10.2	81.4 ± 8.0	62.4 ± 3.5
	B	862.0 ± 10.5	77.2 ± 3.5	60.8 ± 5.0
	C	914.0 ± 11.6	63.2 ± 7.3 ***	22.2 ± 6.3 ***
	D	940.2 ± 10.0	51.7 ± 10.9 ***	8.1 ± 3.6 ***

***$p < 0.001$

A. Control; B. Saline; C. 0.5 ppm $CdCl_2$; D. 1.0 ppm $CdCl_2$

▲ M ± S.D. of 5000 hepatic cell counts from 5 embryos per group

Table 6. The labelling index (o/oo) in the mesonephros (Air chmaber injection method)

Days	Grain count / Groups	0-2	3-9	≥10
5	A	587.0 ± 46.2◆	228.8 ± 20.5	184.2 ± 15.4
	B	638.0 ± 37.0	196.0 ± 26.2	166.0 ± 16.6
	C	784.0 ± 30.8	130.9 ± 12.6***	84.9 ± 18.9 ***
	D	783.6 ± 49.6	153.7 ± 31.1***	62.7 ± 9.6 ***
6	A	827.7 ± 67.2	107.3 ± 25.1	65.0 ± 11.1
	B	844.9 ± 29.2	97.6 ± 27.8	57.5 ± 15.5
	C	868.7 ± 49.2	82.9 ± 11.0***	48.4 ± 6.6 ***
	D	911.2 ± 48.9	47.0 ± 15.0***	41.8 ± 11.7 ***
7	A	903.2 ± 11.9	51.5 ± 8.4	45.3 ± 3.7
	B	900.3 ± 14.3	58.1 ± 9.9	41.6 ± 6.0
	C	907.0 ± 12.9	54.9 ± 9.0	38.1 ± 4.4 ***
	D	924.6 ± 16.7	41.5 ± 10.5***	33.9 ± 4.5 ***
8	A	930.0 ± 10.5	47.0 ± 7.6	23.0 ± 6.3
	B	932.0 ± 5.6	45.8 ± 6.9	22.1 ± 3.6
	C	958.1 ± 11.5	34.2 ± 9.1***	6.4 ± 2.4 ***
	D	971.2 ± 5.0	26.6 ± 4.5***	2.7 ± 1.9 ***

*** $P < 0.001$

A. Control; B. Saline; C. 0.05 ppm $CdCl_2$; D. 1.0 ppm $CdCl_2$

◆ M ± S.D. of 5000 cell counts from 5 embryonic mesonephros per group

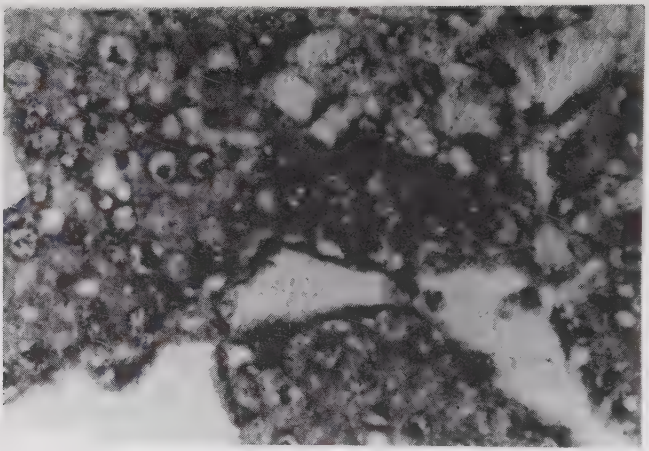


Fig. 6.: Autoradiography of the 5-day control liver (860x).

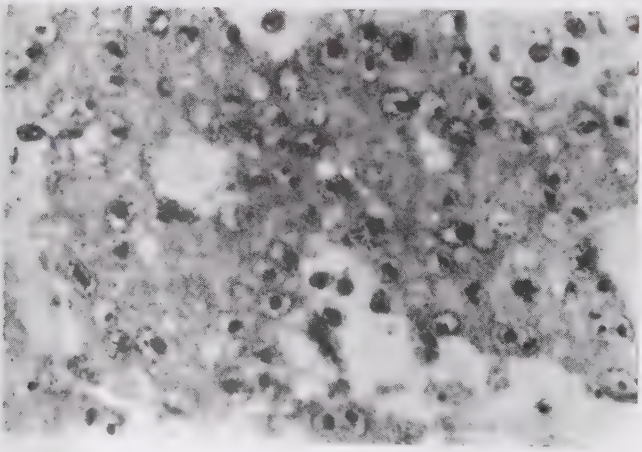


Fig. 7.: Autoradiography of the 5-day liver treated with 1.0 ppm $CdCl_2$ in the air chamber. The amount of tritiated thymidine incorporated in hepatocytes is less than the control (860x).

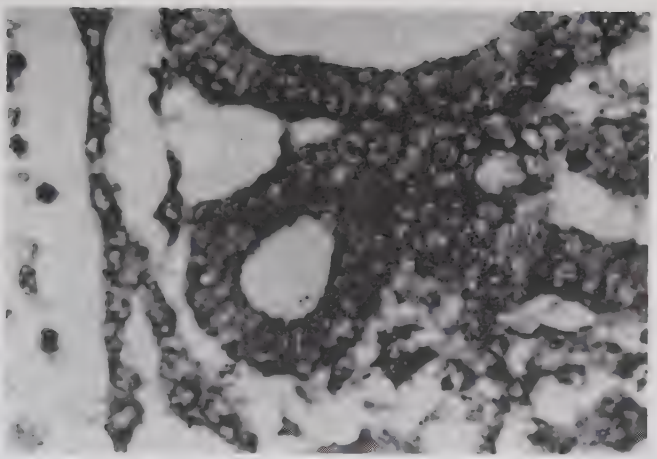


Fig. 8.: Autoradiography of the 5-day control mesonephric tubules (860x).

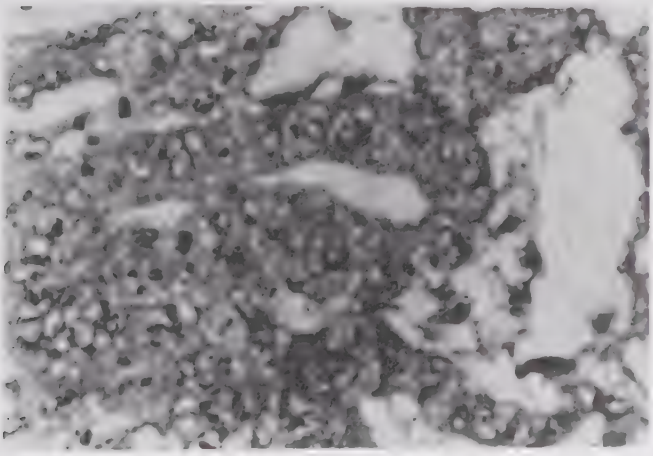


Fig. 9.: Less amount of tritiated thymidine incorporated in the tubular cells of the 5-day mesonephros treated with 1.0 ppm $CdCl_2$ in the air chamber (860x).

Fig. 10. The labelling index in the liver of the chick embryo

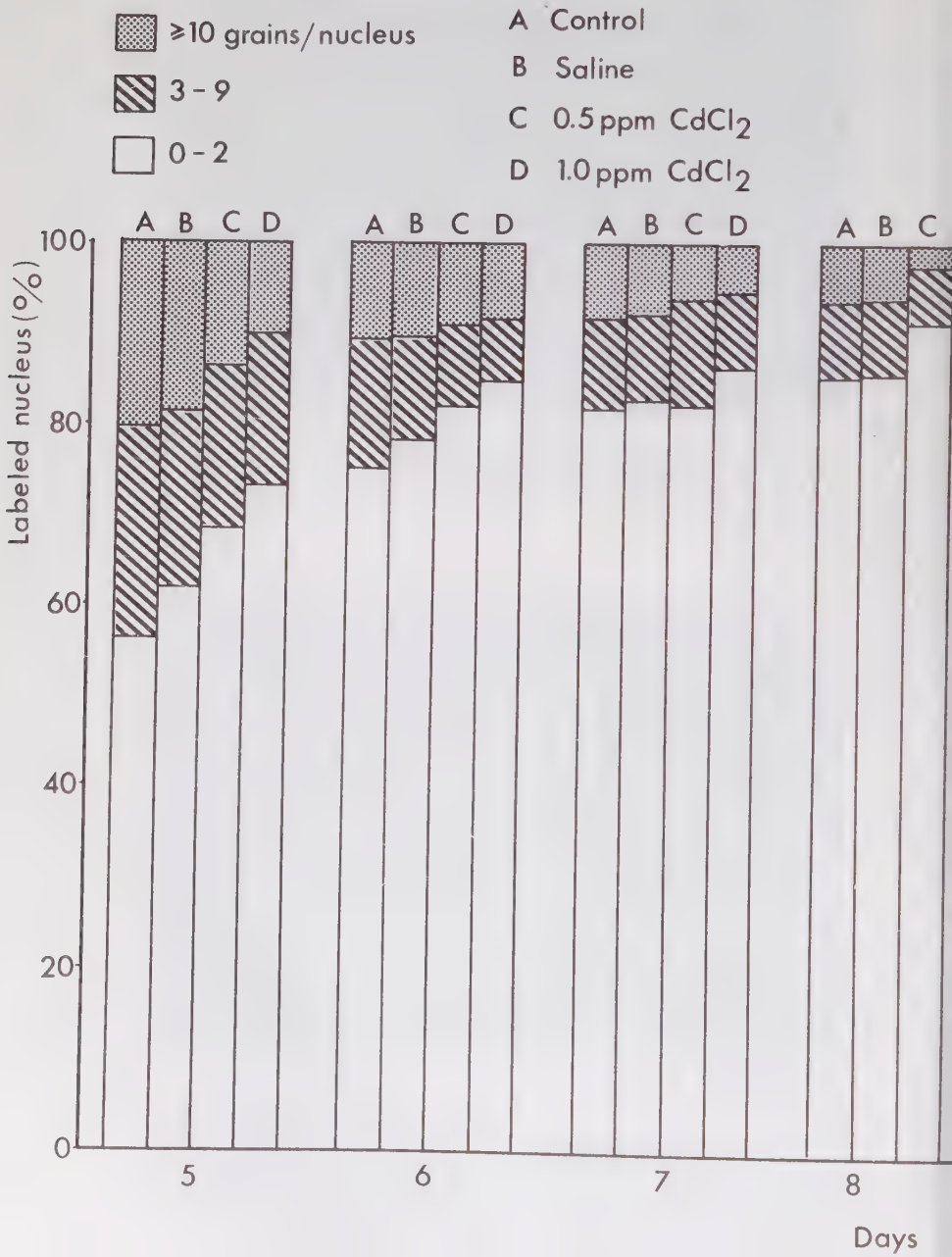
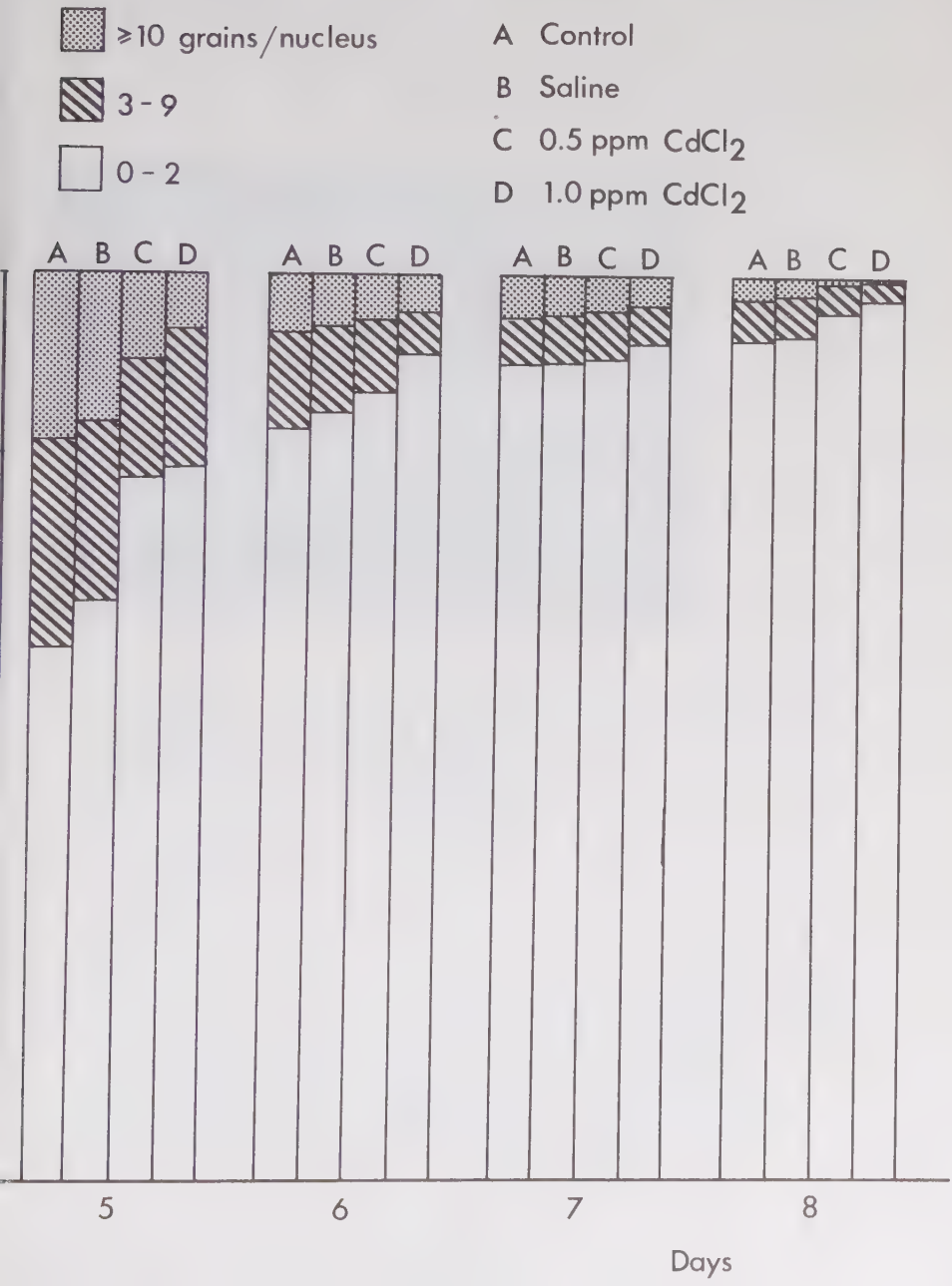


Fig.11. The labelling index in the mesonephros of the chick embryo



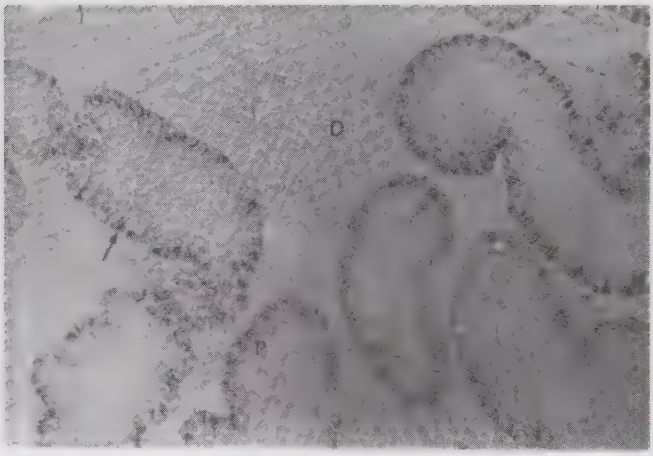


Fig. 12.: Trypan blue granules (arrows) localize at the base of the proximal tubular cells (P) from a 8-day control mesonephros. No trace of trypan blue granule can be found in the distal tubules (D) (340x).

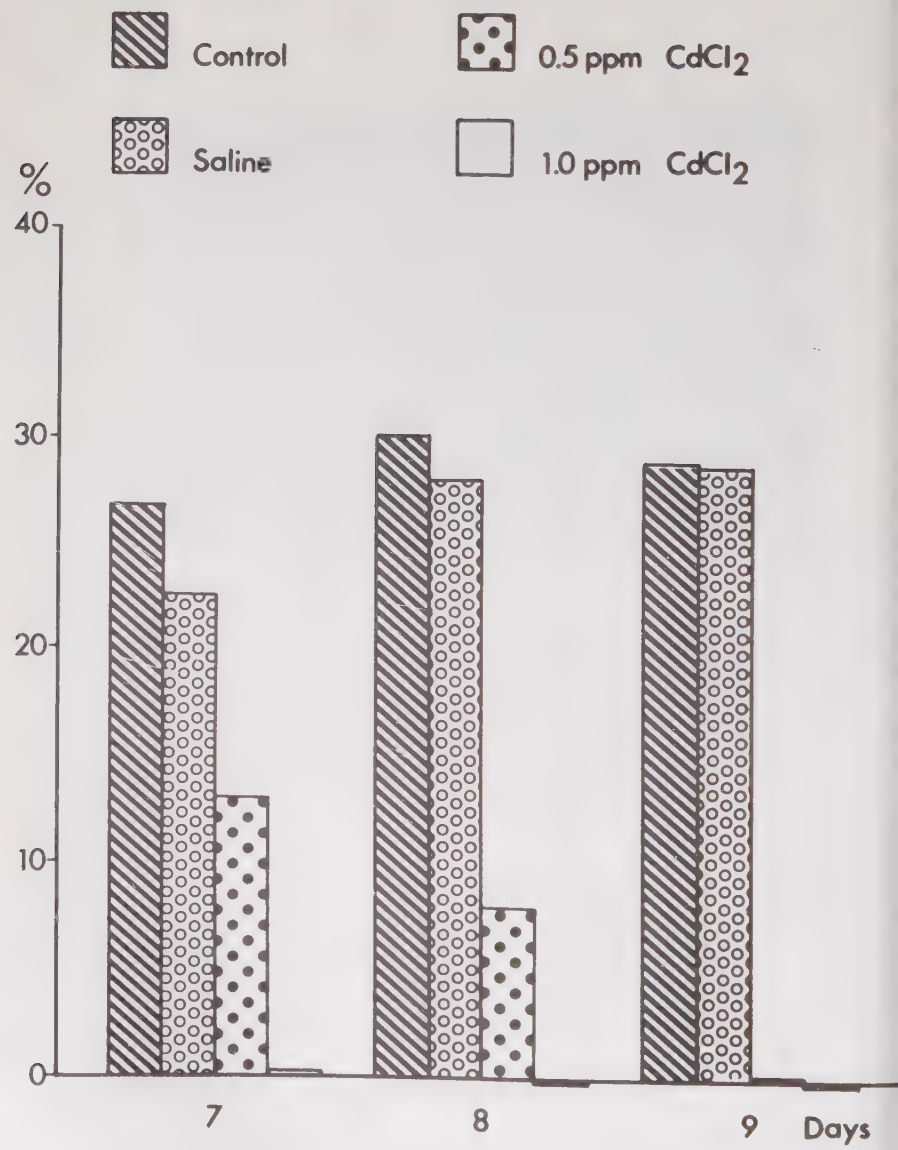
Table 7. Trypan blue index (%) in the proximal tubules of the mesonephros

Groups \ Days	7	8	9
Control	26.5 ± 3.0●	30.1 ± 2.8	28.8 ± 2.7
Saline	22.6 ± 1.9	28.1 ± 2.6	28.7 ± 3.4
0.05 ppm $CdCl_2$	12.9 ± 1.9 ***	7.9 ± 1.5***	-
1.0 ppm $CdCl_2$	-	-	-

*** $p < 0.001$

● The index is based on 5000 counts from 5 embryos per group.

Fig. 13. Mesonephric trypan blue index (Air chamber method)



B. Window injection method

1. Survival rate and body weight

30 eggs in each group were used to measure the survival rate in the window injection method. The analysis of the mortality rate shows that the effects are dependent upon the day of injection and the cadmium concentration ($p < 0.001$)(Table 8).

The survival rate using the window method was lower than that from the air chamber injection method. The vehicle controls (Locke's solution controls) had a 30% - 50% mortality. No single treated group had a survival rate of more than 50%, and all the embryos died from the 8th day's administration of cadmium. It is noted that 0.1 ppm $CdCl_2$ was too high a concentration for the embryos thereby giving rise to only 15% survival rate on the 5th day and subsequently death to all the embryos (Fig. 14).

All the experimentals in the window injection method showed a significant difference ($p < 0.001$) in the body weight when compared with the Locke's solution controls (Table 9). Embryos from the 0.05 ppm groups had an average body weight only half of that of the controls.

2. Gross morphology and histological observation

a. Gross morphology

The abnormalities of the defected embryos by window injection of cadmium solution can be easily recognized, e.g. the developmental retardation of the entire body and organs, changes of the shape, etc. (Fig. 15). In the severely damaged embryos, the lack of eyes, limbs or even problems in the identification of topographic relation of the organs occurred (Fig. 16).

Table 8. The survival rate (%) of the chick embryos (Window injection method)

Groups \ Days	5	6	7	8
Control	100[a]	100	88	75
Locke's solution	70	57	54	50
0.01 ppm $CdCl_2$	50 (25)	46 (40)	25 (50)	0
0.05 ppm $CdCl_2$	40 (40)	38 (60)	24 (80)	0
0.1 ppm $CdCl_2$	15 (40)	0	0	0

Figures in the parenthesis are the percentage of the alive embryos with gross morphological changes

[a] The survival rate is based on 30 embryos per group.

Fig.14. Survival rate of the chick embryos (Window injection method)

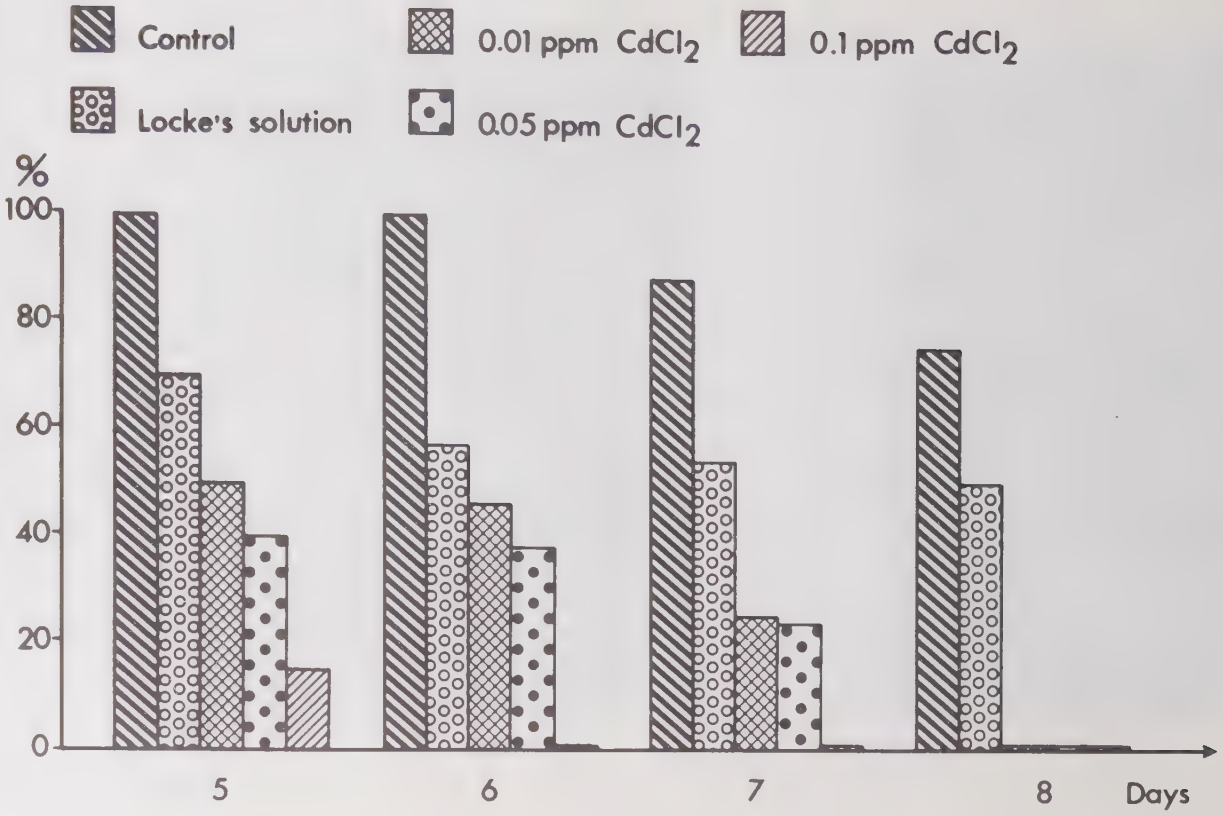


Table 9. The body weight of the chick embryo (Window injection method)

Groups \ Days	5	6	7	8
Control	0.11 ± 0.04●	0.27 ± 0.08	0.43 ± 0.20	0.94 ± 0.22
Locke's solution	0.10 ± 0.04	0.23 ± 0.06	0.42 ± 0.17	0.94 ± 0.21
0.01 ppm $CdCl_2$	0.06 ± 0.02***	0.18 ± 0.05**	0.28 ± 0.08***	-
0.05 ppm $CdCl_2$	0.01 ± 0.00***	0.10 ± 0.01***	0.25 ± 0.04***	-
0.1 ppm $CdCl_2$	0.09 ± 0.00***	-	-	-

** $p < 0.01$

*** $p < 0.001$

● 30 embryos were employed in each group.

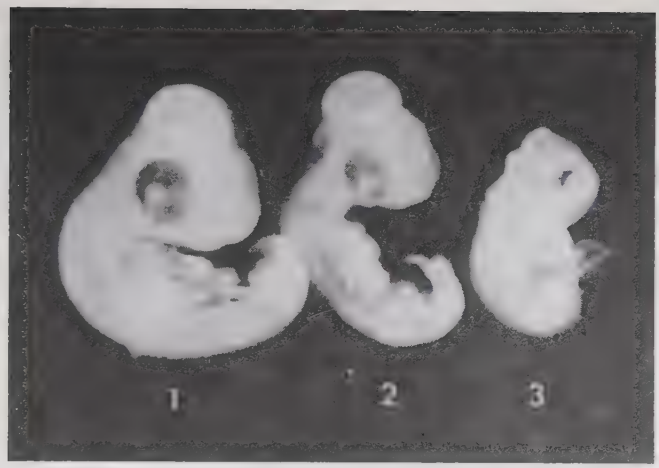


Fig. 15.: A comparison of the 7-day embryos from the air chamber method (2), window method (3) and the control (1) (6x).

Fig. 16.: Completely malformed 7-day embryos from the window method. Embryo with eyes (left, arrow) or without eye (right) but no body shape can be recognized.

b. Histological observation

The histological studies were mainly based on the examinations of the liver and the mesonephros. The spinal cord, eyes and the pancreas have also been investigated.

Spinal cord

The spinal cord was one of the most affected parts of the embryo when compared to the control (Fig. 17). Severe changes or destruction of the spinal cord were noted from the embryos with gross abnormalities. An irregular construction of the neural tube (Fig. 19), decreased thickness of the lateral wall (Fig. 20) or opened dorsal portion of the tube could be observed in the embryos from the different experimental groups.

Eyes

No dose-effect could be related to the eyes. Similar defects of the eye could be found in the embryos receiving different levels of cadmium. Administration time did not play an important role. In some specimens, cup formation was almost abolished and a vesicle with only pigment was present (Fig. 18). On the other hand, some eyes with well developed optic cups contained no pigment. Reduced size of the eye, the optic cup or the lens were common features, and some of the embryos possessed only one eye or even none.

Pancreas

The degeneration of the acini and the islets were another characteristic of the cadmium treated embryos. In the severely malformed embryos, some of the pancreatic cells showed clumping (Fig. 22).

6. ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurden 20 Konzentrate der Gerinnungsfaktoren II, VII, IX und X (PPSB) untersucht. Es stellte sich heraus, daß bei sehr vielen Präparaten die Herstellerangaben über die Faktorenaktivitäten unvollständig, mangelhaft oder sogar unrichtig waren. Ebenso entsprachen die Relationen der Gerinnungs-Faktoren untereinander nur in wenigen Konzentraten der des Normalplasmas. Besonders auffällig war, daß F VII in den meisten Präparaten niedrig angereichert ist, was wegen seiner ohnehin sehr kurzen Halbwertzeit von ca fünf Stunden entscheidende therapeutische Konsequenzen für den Einsatz von PPSB-Konzentraten hat.
Anhand der spezifischen Aktivität wurde der prozentuale Anteil an gerinnungsaktivem Protein bestimmt. Hoher gerinnungsinaktiver Eiweißanteil kann für die durch diese Präparate gelegentlich ausgelösten allergischen Reaktionen verantwortlich sein, was mit der Bestimmung des Denaturierungsgrades erfaßt wurde. Schließlich wurde der Gehalt des einigen Präparaten zur Vermeidung potentieller thrombogener Reaktionen zugesetzte Antithrombin III ermittelt. Keines der untersuchten PPSB-Konzentrate enthielt jedoch genügend AT III, um - auch nach Heparin-Zusatz - eine in-vitro-Gerinnung verhindern zu können.

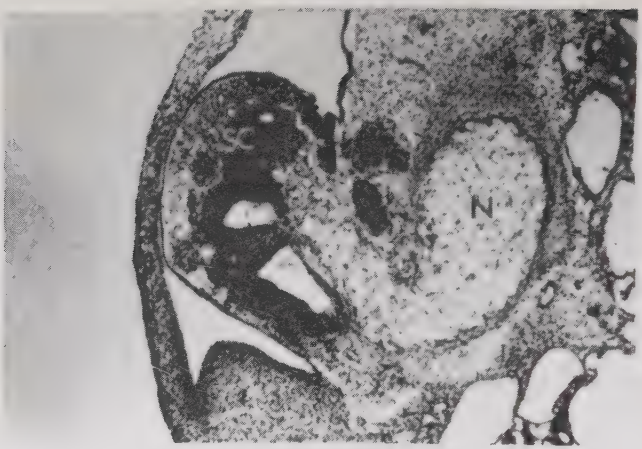


Fig. 19.: A cross section of an irregularly formed spinal cord (SC) from a 7-day embryo treated with 0.05 ppm $CdCl_2$ by the window method. Notochord (N) is also abnormal (85x).

Fig. 20.: 0.05 ppm $CdCl_2$ treated 6-day embryo from the window method with unevenly thick lateral wall of the spinal cord (arrow) (85x).

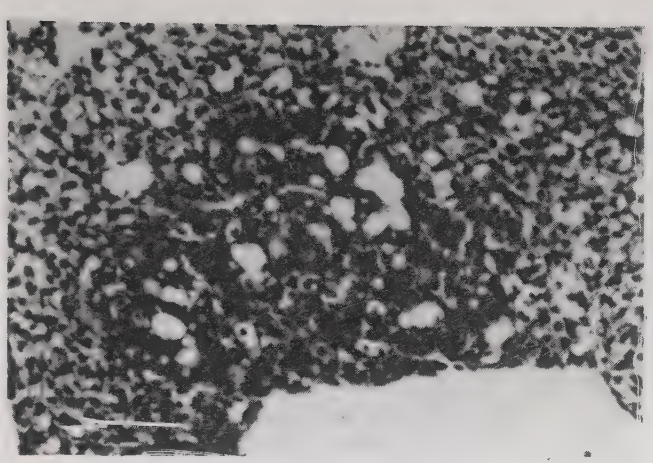


Fig. 21.: Pancreas of the 7-day control embryo (340x).

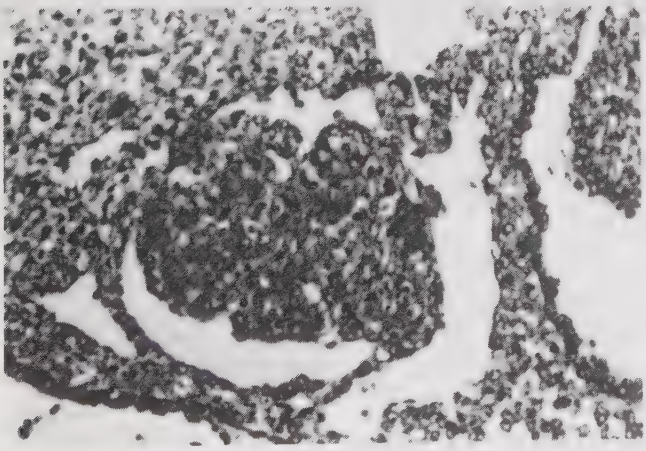


Fig. 22.: A cross section of the 7-day pancreas treated with 0.05 ppm $CdCl_2$ by the window method shows the degeneration of the acini and the islets (340x).

Liver

A retarded liver with dilated sinusoids (Fig. 24) was found to be the common features in all the cadmium treated embryos. From the 5th incubation day, the hepatocytes showed hypertrophy, with changes in the normal structure of the hepatic cords. Hepatocellular necrosis was common. Within the cells, the vacuolization of the cytoplasm and the swelling of the nucleolus were prominent. Along the hepatic cords, the Kupffer cells transformed from spinal to oval shape. Similar pattern was observed from the 6-day incubation group of livers.

Absence of hepatic cords, dissociation of the hepatocytes (Fig. 25), cellular disintegration or degeneration were the main features of the 7th day liver.

Mesonephros

In the mesonephros of all the malformed embryos, the renal corpuscle showed either abnormal shrinkage or enlargement (Fig. 28). The glomeruli were enlarged with a dilated lumen of capillary tufts and a widened space of Bowman. But within most of these enlarged glomeruli, the size of the capillary tufts was particularly small or even degenerated. In some cases, several tubules shared a common glomeruli.

Necrotic or degenerative cells were found in the enlarged convoluted tubules (Fig. 29). In the 7th day incubation group, corpuscles and desquamated cells were found in the lumen of the tubules.

3. Enzyme histochemistry

Two enzymes, namely ACPase and LDH, were investigated in the embryos from the window injection method. The results of activities of the controls have been described in section A. 3.

Fig. 23.: A cross section of the liver from the 7-day control embryo (85x).

Fig. 24.: A cross section of a 5-day embryo treated with 0.05 ppm $CdCl_2$ by the window method shows the enlarged sinusoids and the destructed hepatic cords (85x).

Fig. 25.: A cross section of a 7-day embryo administered with 0.05 ppm $CdCl_2$ through the window of the shell shows the disintegration of the cells from the hepatic cords (340x).

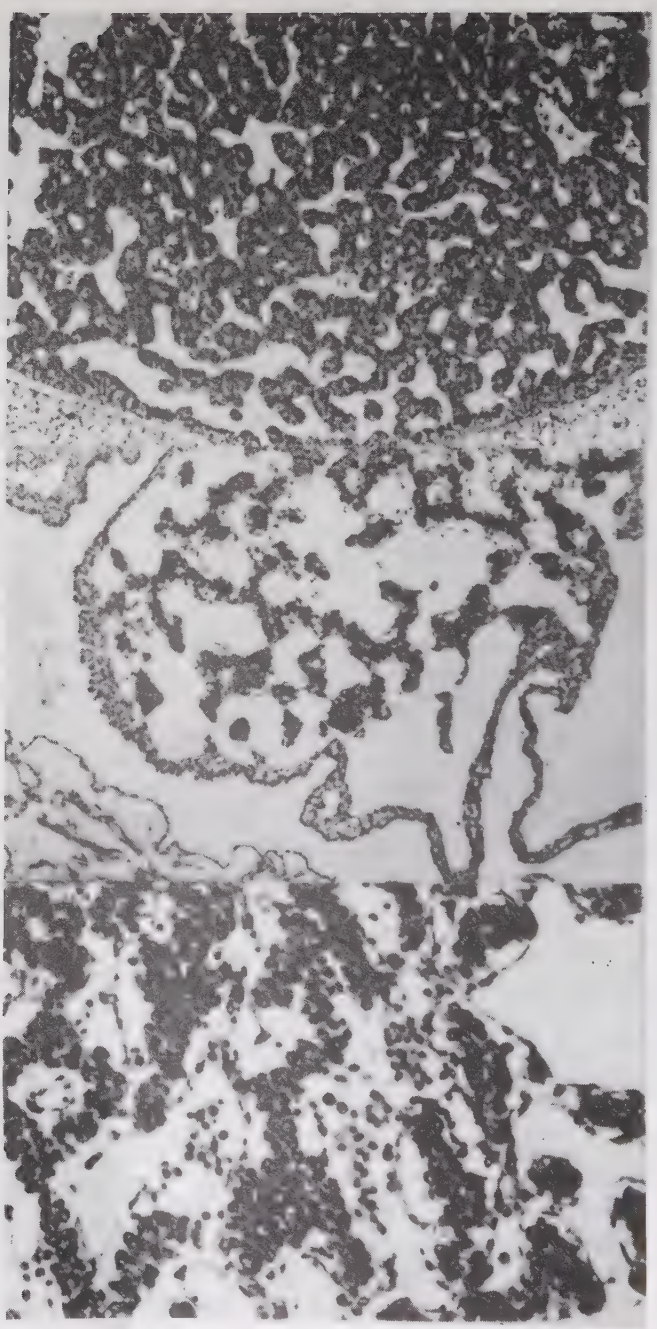


Fig. 23

Fig. 24

Fig. 25

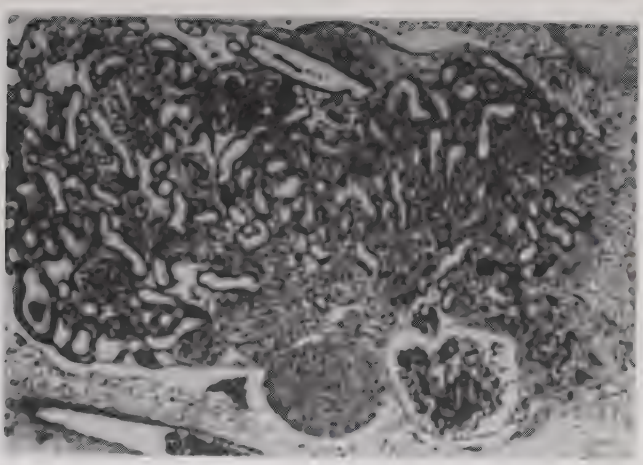


Fig. 26.: A longitudinal section of the 7-day control mesonephros (85x).

Fig. 27.: Enlarged tubules (arrows) in the mesonephros of a 7-day embryo treated with 1.0 ppm $CdCl_2$ in the air chamber (85x).

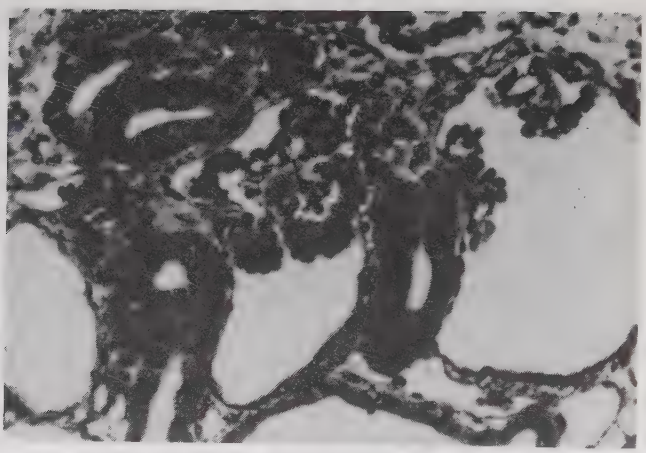


Fig. 28.: Cross section of renal corpuscles from a 7-day embryo treated with 0.05 ppm $CdCl_2$ through the window of the shell shows the enlarged Bowman's capsule and shrinked glomerular capillary tufts (340x).

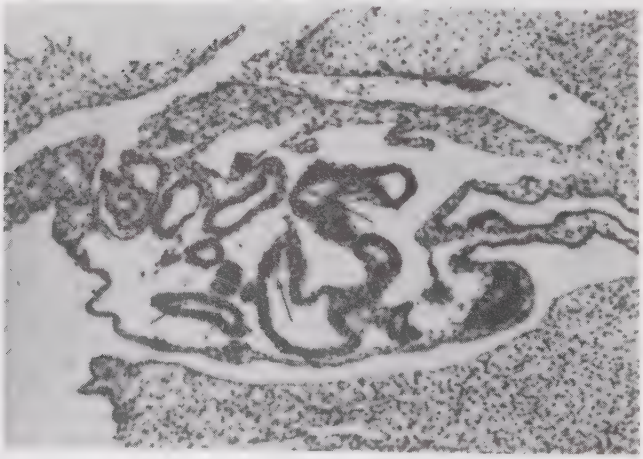


Fig. 29.: Limited amount of enlarged tubules and glomeruli in the 7-day mesonephros treated with 0.05 ppm $CdCl_2$ through the window of the shell. Corpuscles and desqumated cells (arrows) can be observed in the tubules (85x).

a. Acid phosphatase (ACPase)

Generally, the liver of the chick embryo after cadmium administration showed a decrease in enzyme activity (Fig. 31). The ACPase changed from fine granules in the control to large granulated arrangement in the cytoplasm of the treated parenchyma.

The ACPase, appeared as large granules in the cytoplasm and showed a considerable increase of activity in the mesonephric tubules of the cadmium treated embryos. Necrosis was found in the glomeruli, and glomerular enzyme activity became more and more concentrated as the cadmium level was elevated (Fig. 33).

b. Lactic dehydrogenase

Compared to the cup-shape arrangement in the cytoplasm of the parenchyma, the LDH of the experimentals was in dots or small spheres, and the activity decrease regularly in the whole cytoplasm of the parenchyma (Fig. 35).

The mesonephros of the chick embryo had higher LDH activity in the tubular cells than did the controls. The enzyme scattered in the apical region or along the luminal border appeared in large granules, suggestive of mitochondrial swelling (Fig. 37).

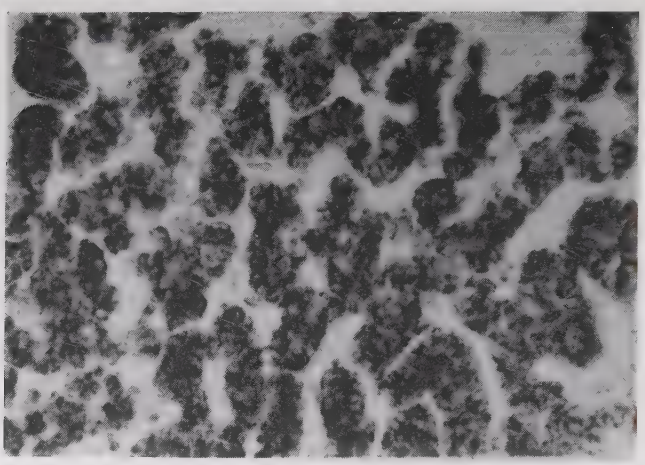


Fig. 30.: Acid phosphatase activity in the liver of the 5-day control embryo (340x).

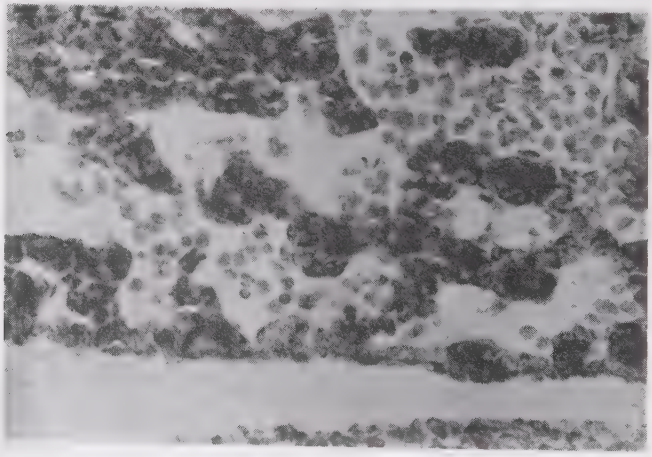


Fig. 31.: Acid phosphatase activity in the liver of the 5-day embryo treated with 0.05 ppm $CdCl_2$ supravitellinely (340x).

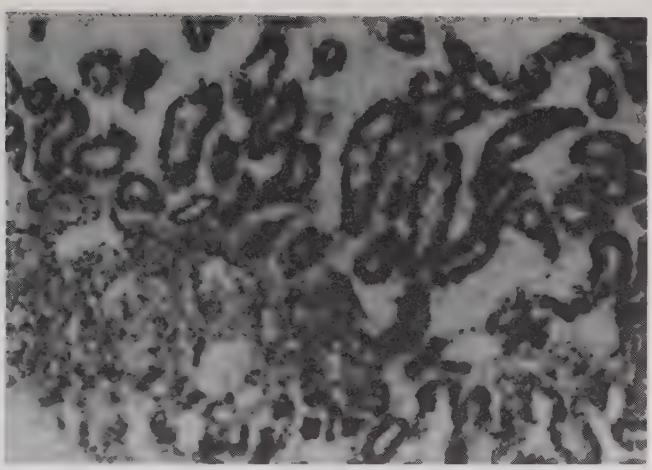


Fig. 32.: Acid phosphatase activity along the luminal border of the tubular cells in the 8-day control mesonephros (140x).

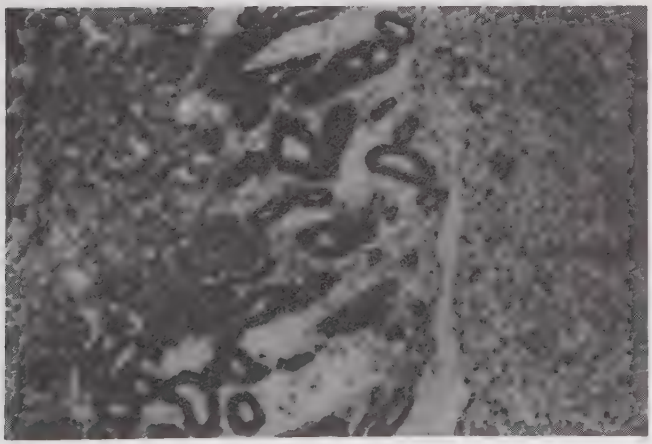


Fig. 33.: Increased acid phosphatase activity in the tubular cells in the 8-day mesonephros treated with 0.05 ppm $CdCl_2$ supravitellinely (140x).

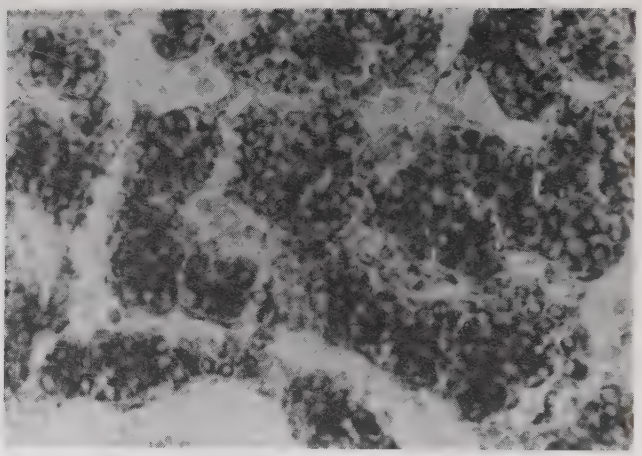


Fig. 34.: LDH activity in the liver of the 5-day control embryo (340x).

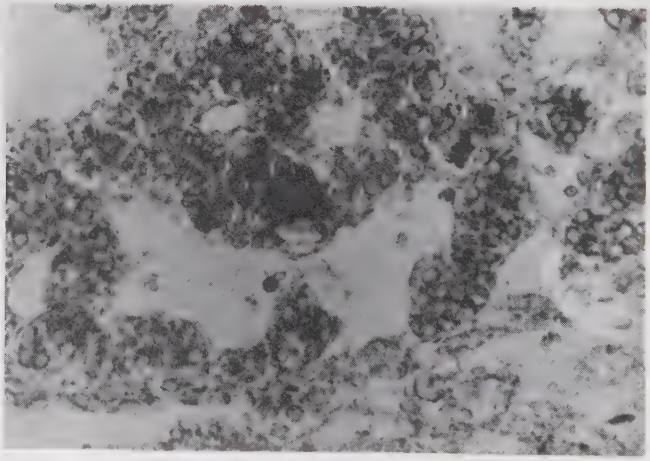


Fig. 35.: Activity of LDH in the liver of the 5-day embryo treated with 0.05 ppm $CdCl_2$ supravitellinely (340x).

Fig. 36.: LDH activity in the mesonephros of the 5-day control embryo. Arrow shows a proximal tubule (340x).

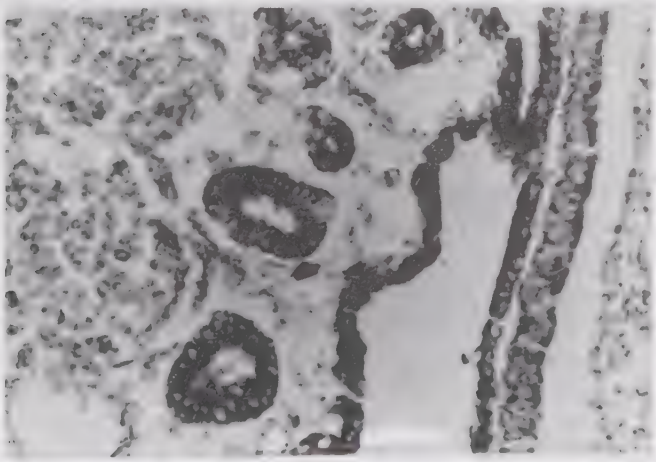


Fig. 37.: Increased LDH activity in the proximal tubules (arrow) of the 5-day mesonephros treated with 0.05 ppm $CdCl_2$ supravitellinely (340x).

IV. Discussions

Cadmium effects in the air chamber injection method

The decreased survival rate of chick embryos after administration of $CdCl_2$ solution in the air chamber shows that cadmium is highly toxic to avain embryos. The 8 - day survival was only 44% and 21% in the 0.5 ppm and 1.0 ppm cadmium treated groups respectively. However, the survival rate of embryos from the 0.1 ppm $CdCl_2$ treated groups shows no great difference to that of the controls. This indicates that 0.1 ppm cadmium chloride is too dilute a concentration in the air chamber injection method to cause any remarkable effects on the embryo. As far as the body weight is concerned, no difference exists between the embryos with 0.1 ppm $CdCl_2$ and the controls. On the other hand, a significant decrease of the body weight was observed in those groups with higher cadmium dosages, i.e. 0.5 ppm and 1.0 ppm $CdCl_2$. This phenomenon is well explained by the mitotic index in organs. Mitotic index is an useful tool for the comparison of the proliferative activity of the organ or the whole organism. There was about 20% and 25% decrease in mitoses in the liver and the mesonephros respectively of the 0.5 ppm $CdCl_2$ treated groups, and 30% and 45% decrease in the 1.0 ppm groups during the 5th to 8th incubation days. According to Bisceglie (1931), the maximal mitotic activity of the liver and the mesonephros occurs around the 5th day of incubation, and the depression of mitotic activity, especially in this critical period will be an important retarding factor for the normal development of the chick embryo. As a result of the decrease of the mitosis in the organs, the body weight of chick embryos, particularly those from the 1.0 ppm cadmium treated groups, decreases significantly.

The findings using the labelling index correlated with the mitotic or cell proliferative activity. The labelling index from the incorporation of the radioactive thymidine decreases sharply among those cadmium treated groups implies that cadmium hinders one or more steps during mitosis. Webb et al. (1972) found 50% of radioactive cadmium injected can be traced in the nucleoli of the embryo and the remainder in the nuclear sap and the chromatin. This nuclear uptake of metal and their incorporation into the nucleic acids during growth makes it possible to affect the conformation and biological activity of both DNA and RNA, and consequently the replication, transcription and translation. Stoll et al. (1976) suggested that cadmium interacts directly with DNA by changing its melting profile. Kacew et al. (1976) argued that cadmium does not inhibit DNA synthesis directly but regulates the deoxynucleic acid metabolism through the modulation of the cyclic AMP-protein kinase system. Inhibition of RNA polymerase activity and consequently the protein synthesis and mitosis are found in the nuclei exposed to cadmium (Hidalgo et al., 1976; Sina and Chun, 1978, 1979). Hidalgo et al. (1976) also found a decrease of cytoplasmic protein synthesis, which is independent of the effect of the metal on RNA synthesis. This is an indirect effect from the disturbance of oxidative phosphorylation in the mitochondria under the cadmium treatment that no enough energy affords the protein synthesis and mitosis (Jacobs et al., 1956; Fluharty and Sanadi, 1962; Cherian et al., 1976).

The results from the autoradiographic study coincide the inhibition of DNA synthesis suggestion that in the developing liver and mesonephros, the higher dose of cadmium administered, the less labelled thymidine is incorporated in the chromosome or precisely in the DNA. On the

other hand, evidence of the change of mitochondrial structure from the histochemical observation of LDH also provides the possibility that the disturbance of energy supply in the affected mitochondria is one of the inhibiting factors of the thymidine incorporation in the newly sythesized DNA. With the hindered replication of DNA by the action of cadmium, the cell proliferation is depressed, which gives rise to a lower body weight or even the lethality of the embryos.

Trypan blue has been used as a vital dye to study renal functions since the beginning of this century (Evans, 1914; Hanan, 1927; Hurd, 1928). However, it also causes teratogenic effects, including cellular necrosis, retardation of organs and whole body development or even mortality (Hurd, 1928; Beaudoin and Wilson, 1958). Embryos treated with cadmium solution in the air chamber show no distinct histological changes in the mesonephros compared to the controls, but appear to have lost their ability to retain trypan blue granules in their proximal tubular cells.

In the chick embryo, mesonephroi differentiate on the 2nd day and start functioning on the 5th day (Freeman and Vince, 1974). When the trypan blue solution is injected into the air chamber of the egg, it will reach the embryo through capillaries of the chorio-allantois, and is absorbed by the proximal tubules. No granules can be found in the mesonephroi of embryos after incubated with 0.5 ppm $CdCl_2$ for 8 days or those from all the 1.0 ppm $CdCl_2$ groups. This is evidence of the loss of normal function of the mesonephros after cadmium treatment. However, it should be noted that trypan blue itself is a teratogen, and thus may enhance the toxicity of cadmium on the mesonephros.

Injection methods

It is interesting to note that analysis of the mortality rate does show a significant effect of the cadmium on chick embryos, and the difference in the body weight, mitotic and labelling indices or even the mesonephric functional test by trypan blue are consistent this. However, no gross morphological and histological changes could be observed in the experimentals after the administration of cadmium in the air chamber, except that some of the embryos from the 1.0 ppm group after 7 days of incubation had alterations in their mesonephroi and livers.

The cadmium content of the embryos as determined by atomic absorption spectrometry is shown in Table 10. A great variation of cadmium content can be found in embryos from the same treatment group. Embryos with a considerable amount of cadmium in the body died. On the other hand, those alive and with a relatively high body weight possessed a low cadmium content. Some of them even had the same cadmium levels as the controls. That suggests that those embryos which absorbed the cadmium injected into the air chamber died. Those are still alive had actual cadmium administration of far less that 0.5 ppm and 1.0 ppm $CdCl_2$. There are two possible causes of this variation in embryo cadmium content:

1. The cadmium solution, once injected into the air chamber, has to pass the inner shell membrane and diffuse in the albumin before reaching the vitelline membrane and the blastoderm. During this long way from the air chamber to the embryo, part of the cadmium will be absorbed by the inner and the outer membranes and the vitelline membrane. It will also be diluted in the albumin. The dilution depends on the egg size and age. Some of the eggs

Table 10. Cadmium content (ng Cd/embryo) in the chick embryo (Air chamber method)

Groups	Egg no.	Incubation days			
		7	8	9	10
Control	1	2.0	<1.0	1.5	1.9
	2	<1.0	<1.0	1.8	<1.0
	3	<1.0	<1.0	1.2	<1.0
	4	<1.0	2.1	1.5	<1.0
	5	1.7	<1.0	<1.0	1.5
Saline	1	<1.0	<1.0	1.8	<1.0
	2	3.3†	<1.0	1.4	1.1
	3	<1.0	<1.0	2.0	1.7
	4	<1.0	<1.0	1.8	1.4
	5	1.5	<1.0	<1.0	<1.0
0.01 ppm $CdCl_2$	1	<1.0	<1.0	9.9†	<1.0
	2	6.3†	3.1†	4.4†	<1.0
	3	2.0	1.3	3.0	<1.0
	4	<1.0	1.3	3.5†	<1.0
	5	<1.0	<1.0	1.4	<1.0
0.1 ppm $CdCl_2$	1	<1.0	1.3	2.5	1.3
	2	<1.0	<1.0	11.1†	2.8†
	3	2.1	<1.0	3.4†	1.7
	4	2.6	<1.0	1.9	1.2
	5	<1.0	1.1	1.3	<1.0
1.0 ppm $CdCl_2$	1	6.0†	7.3†	1.8	14.0†
	2	<1.0	12.8†	1.1	25.5†
	3	<1.0	14.0†	3.5†	69.6†
	4	7.1†	3.9†	2.7	29.8†
	5	<1.0	1.3	23.3†	15.0†

†Dead embryo

have already started to develop in the hen before they are laid (Huettner, 1953). Water in the albumin will partly evaporate or partly be absorbed by the embryo. By the air chamber injection method, it is impossible to observe the embryo during Cd administration, nor determine its developmental stages. Absorption and dilution are thus two undeterminant factors for the calculation of the effective dose of Cd in the embryo.

2. Cadmium not only has a very high affinity for a low molecular weight protein - Metallothionein (Margoshes and Vallee, 1957), but also can react with a variety of proteins (Vallee and Ulmer, 1972). Cadmium binds firmly to coalbumin (Tan and Woodworth, 1969). It competes with other metals, like Zn, Cu and Ni, for the same Sulfhydryl or imidazole groups in human and other animal protein (Perkins, 1961). Metallothionein has been identified in the chicken liver (Weser et al., 1973) and chick duodenum (Starcher, 1969), but no information is available concerning the chick embryo, especially during the early stages of development.

Feeney and Allison (1969) found that 90% of the total weight of the albumin is water and over 90% of the solids in the albumin are proteins. Most of them are ovoalbumin (54%), similar quantity of conalbumin and a small proportion of ovomucoid (App. 1). It is possible that part of the cadmium ions will bind to the albumin proteins. Research suggests that cadmium toxicity is mediated by intracellular actions of the ions and thus protein-binding reduces cadmium toxicity (Yoshikiwa et al., 1973, 1974).

Regarding the injection method, Karnofsky, one of the pioneers in using the chick embryo to screen drug effects, suggested that injecting test compounds in the yolk sac

Appendix 1. The percentage composition of the proteins of egg albumin*

Constituents	Units / gram
Ovotransferrin	12
Ovomucoid	11
Ovoinhibitor	1.5
Lysozyme	3.4
Ovomacroglobulin	0.5
Flavoprotwin	4.7
Ovomucin	2.9
Avidin	11.5

* Data from Feeney & Allison (1969)

on the 4th day of incubation is the most suitable method, because the congenital malformations from the embryo can be seen macroscopically (Karnofsky, 1955), and the problem of exact dosage is solved. However, this method has many disadvantages. The site specificity of a certain drug is closely related to the timing of the organogenesis of the embryo (Ferm et al., 1968). Organs like the mesonephros, which has already developed and is ready to function at the end of the 4th day, is not so susceptible to the drug by this method, and hence some of the major drug effects may be missed. Besides, the yolk sac is not simply a nutrient store for the chick embryo but also functions as part of the gut or acts as a "transitory liver" to synthesize and store glycogen (O'Connor, 1953; Ballard and Oliver, 1963; Perrier, 1964). Many enzymes have been found in the yolk sac membrane (Berg and Szerkerzes, 1962; Bernhard et al., 1973). Once the membrane is damaged by the yolk sac injection, the enzyme system is disturbed, and the inclusions of the yolk will leak into the albumin. Furthermore, the injection of external substance into the yolk sac will cause a stress to the developing embryo. Birge and Roberts (1976) have reported the survival rate of the chick embryo after cadmium injection on the 1st day of incubation into the yolk sac. The LD_{50} is found at about 0.05 ppm cadmium ion (App. 2). By this route, cadmium is far more toxic than with the air chamber injection.

Besides these two injection methods, test substances are also injected into the allantois (Robertson et al., 1964); the amnion (Williamson et al., 1967) and the vein (Lee, 1946). All these methods can only be carried out after the 4th incubation day and also have a very high mortality rate of the embryo due to the damage of tissues and organs.

Appendix 2. The survival rate of chick embryos (Yolk sac injection)*

Concentration (ppm)		Percent survival
Cd^{++}	$CdCl_2$	
0.001	0.0016	66
0.010	0.0160	55
0.050	0.0800	48
0.100	0.1600	41 (6)
0.500	0.8000	25 (15)
1.000	1.6000	8 (33)
5.000	8.0000	0
10.000	16.0000	0

* Data from Birge & Roberts (1976)

Percentages of surviving embryos possessing gross teratologies are given parenthetically.

In order to minimise all the above factors, cadmium was given directly on the vitelline membrane, underneath which the embryo is located, through a window in the shell. The vitelline membrane remained intact because an opened vitelline membrane during the first few days of development affects the normal growth of the embryo (New, 1959; Bellairs and New, 1962). Half of the embryos died after administration of 0.01 ppm $CdCl_2$ for 5 days. Only 15% of embryos were alive in the 0.1 ppm $CdCl_2$ treated group on the 5th day, and subsequently all embryos died by the 6th day. This is in striking contrast to the results from the air chamber injection method where the 0.1 ppm $CdCl_2$ is too dilute a dose to cause any histological and morphological changes, and the mitotic and labelling index as well as the enzyme histochemical activities are similar to the controls.

Effects of cadmium in the window injected method

Severe damage in the neural system are found in chick embryos after applying the cadmium chloride solution directly on the 1-day old chick embryo. At this stage of development, the notochord has already developed and the neural fold and head fold appear, but no neural tube can be observed (Hamburger and Hamilton, 1952). Once the $CdCl_2$ solution is applied on the embryo and absorbed by the ectodermal cells, the cadmium ions will inhibit the cell proliferation and retard the neural development. It is not surprising that an opened or unevenly thick neural tube can be observed in the chick embryo.

Mangold (1955) suggested that inducers play a vital role in embryonic development. The neural induction is a triggering process which sets the ectoderm along the path of neural and sensory organs' development, and the eye is one of the best examples. The competent ectoderm under

primary induction of the prechordal plate gives rise the optic cup, which will further induce the formation of the lens (Spemann, 1938). Once the eye cup is destroyed, the lens fails to form. In the chick embryo, the primary optic vesicles arise in the lateral walls of the prosencephalon at about 30 hours of incubation, which is shortly after the administration of the $CdCl_2$ solution. The cadmium ions will directly affect on the developing optic vesicles, as evidence by the necrotic cells within them. This finding is consistent with results from rats following cadmium injection (Krause-Fabricius and Hilscher, 1974). On the other hand, the neural induction for the formation of the eye cup or the secondary optic vesicle is disturbed if the neural development is altered or retarded in the cadmium treated chick embryo. As a consequence, the lens development is also suppressed. In other word, there is a possibility that the malformation found in the eye is not directly from the action of the cadmium, but a secondary product from the damage of the neural system. Evidence for this hypothesis is that those embryos without lens are always accompanied with severely damaged brain and neural tube. The association of defects from both of these organs, like anophthalmia, microphthalmia, encephalocele and exencephaly, are also found in golden hamsters (Ferm et al., 1968).

Pancreas and liver

Pancreatoxicity and hepatotoxicity by cadmium are well known in the research on rats and rabbits, whose ß-cells are damaged (Havu, 1969) or the hepatic carbohydrate metabolism is altered (Singhal et al., 1974). As a result, glucose intolerance, stimulation of gluconeogenesis, and high mortality rate are the common features of the cadmium poisoned animals.

In the chick embryo, the pancreas differentiates on the 3rd day of incubation (Freeman and Vince, 1974), and the ß-cells secrete insulin only in insignificant amount until from the 12th - 13th day (Grillo, 1961; Beaupain and Dieterlen-Liévre, 1972). The liver differentiates on the 2nd day (Freeman and Vince, 1974) but starts to synthesize and store glycogen on the 7th day (Grillo et al., 1964). At the early developmental stages, the yolk sac membrane functions as a "transitory liver" to synthesize and store glycogen. It is therefore interesting to note that instead of disturbing the normal liver functions, cadmium in the liver within the first week of incubation is a general cellular poison, which depresses the DNA synthesis and cell proliferation, increases vacuolization and causes cytoplasmic damages, including the changes of mitochondrial structure and the increase of cell permeability or even the rupture of the cell membrane. The decrease of the enzyme activities of the ACPase and LDH is therefore likely to be lost through the damaged cell membrane. The change of the ACPase and LDH containing particles, namely the lysozymes and mitochondria, from fine granulated form into large granules or other shapes is an evidence of the cellular damage effects of the cadmium in the non-functioning liver. The damage effects of the cadmium on mitochondria and cell membrance have also been found on rats and rabbits (Jacobs et al., 1956; Fletcher et al., 1962; Hoffmann et al.,1975; Christensen, 1975; Cherian et al., 1976; Faeder et al., 1977).

Mesonephros

Although the nephrotoxicity of the cadmium is known over a century (Marmè, 1867), and then countless research reports confirm that the renal damages are caused by cadmium. However, there are different opinions on the mechanism of cadmium toxicity in the kidney. One hypothesis is that the cadmium is transported to the glomeruli and

filtered, and then reabsorbed by the proximal tubules. Therefore, the primary site of cadmium action is considered to be the tubules (Piscator, 1964, 1966; Piscator and Axelsson, 1970; Nordberg, 1972, 1975; Gieske and Foulkes, 1874; Cherian et al., 1976; Takashima et al., 1978). Lauwerys et al. (1976) suggested that the glomerular dysfunction may precede the renal lesion. Several studies on renal damage in humans following cadmium exposure have demonstrated that the renal changes are prominently tubular, but degenerative changes are evident at the glomerular level (Bonnell et al., 1960; Itokawa et al., 1974).

The histological observations in the chick embryo show necrosis and malformations in the tubules and glomeruli. It is possible that the damage of the glomeruli disturbs the normal filtration function and the pressure of the fluid will cause the swelling of the Bowman's capsule and the shrinkage of the glomeruli. Swelling can also be found in the tubules. No finding clearly indicates whether the glomerulus or the tubule is the primary site of the action from cadmium. However, in the damaged embryos from the air chamber injection method, no prominent alteration can be observed in the glomeruli but the swelling of the tubules and the vacuolization of the tubular cells are very common. This finding favors the hypothesis that tubule is the primary site of cadmium action.

LDH and ACPase are not only two enzymes, which can be found in a relatively high quantity in the early developmental stages of the chick mesonephros, they are also proved to be sensitive indicators of damage (Henderson et al., 1979). Increase of LDH activity was reported in response to the metabolic stress from cadmium (Choudhury and Lundy, 1970; Christensen, 1977; Hendersen et al., 1979). Elevation of ACPase activity implying a general lysosomal activation is found in the functioning kidney

of the cadmium treated rabbit (Foster and Cameron, 1963) and other functioning organs in rat (Choudhury and Lundy, 1970; Henderson et al., 1979). Similar increase of LDH and ACPase in the mesonephros of the cadmium poisoned chick embryo is highly consistent with the histological findings that cadmium in the functioning mesonephros is not only an irritant to the mesonephric cells but also causes damage on them, which as a result disturbs the normal function of the whole organ.

In reviewing the studies of the $CdCl_2$ solution on the chick embryo, few histological variations in the liver and the mesonephros have been observed in the air chamber injection method. This may account for the curtailed effective dose of $CdCl_2$ on the embryo by injecting it into the air chamber. However, cadmium does have effects on the mesonephros of the early developmental stages chick embryo as indicated by its functional and histochemical changes. As the cadmium solution is applied more directly onto the embryo like that in the window injection method, its teratogenic effect on the histology of the organs is obvious. From these functional, histological and histochemical investigations, the disturbance of the mesonephric function is possibly the major factor of the early stages chick embryos after the cadmium administration in both the air chamber and the window injection methods.

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Curriculum vitae

I, Wing-keung Liu, was born on 1st March, 1953 in Canton, China, as the last son of the chief accountant, Pak-yuk Liu and his wife, Chi-chu (Dai). In 1959, I emigrated with the family to Hong Kong, where I attended the Sycomore Government Primary School till 1965, and finished my secondary education in Lai Chack Middle School with 5 credits in the Hong Kong Certificate of Education Examination in 1971.

In 1971, I studied Biology and Biochemistry in the Chinese University of Hong Kong and graduated Bachelor of Science with 1st class honours in 1975. From 1975-1977, I studied in the Environmental Biology Division of the Chinese University and achieved my Master of Philosophy.

In June, 1977, I came to Germany and learned the German language in the Goethe Institute, Iserlohn for four months before I was enrolled as a student for the Doctor of Natural Science in the University of Düsseldorf. Prof. W. Hilscher of the Medical Institute of Environmental Hygiene and Prof. G. Willuhn of the Pharmaceutical Biology have been my research advisors.